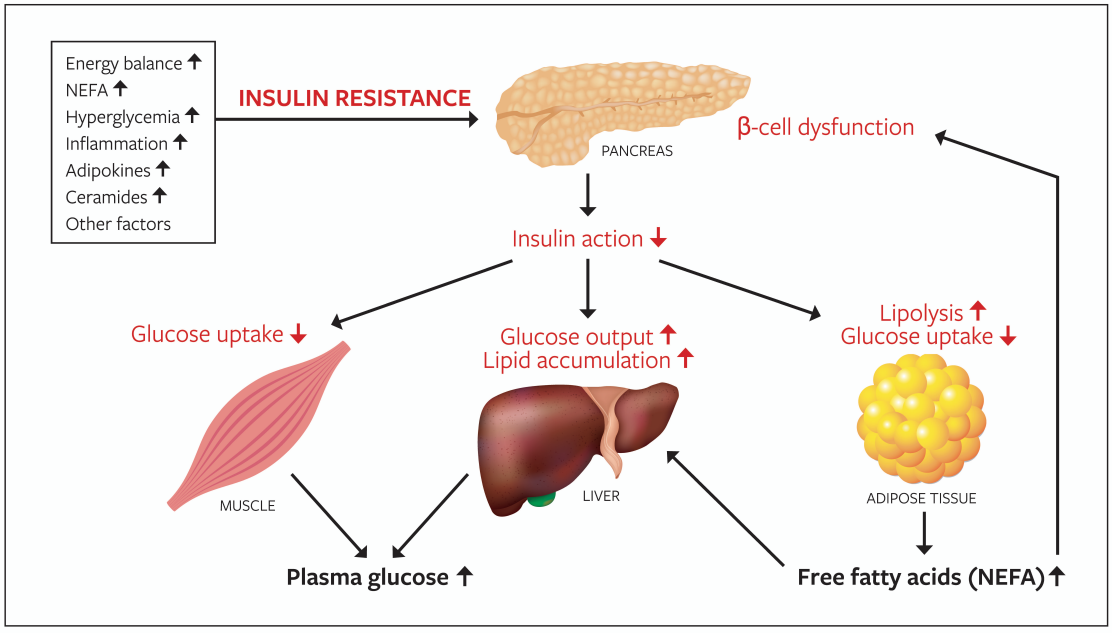
DISSERTATIONES SCHOLA DOCTORALIS SCIENTIAE CIRCUMIECTALIS, ALIMENTARIAE, BIOLOGICAE. UNIVERSITATIS HELSINKIENSIS

SIRU SALIN

EFFECT OF ENERGY ALLOWANCE DURING THE DRY PERIOD ON INSULIN RESISTANCE AND METABOLIC ADAPTATION IN TRANSITION DAIRY COWS ON GRASS SILAGE-BASED DIETS



DEPARTMENT OF AGRICULTURAL SCIENCE
FACULTY OF AGRICULTURE AND FORESTRY
DOCTORAL PROGRAMME IN SUSTAINABLE USE OF RENEWABLE NATURAL RESOURCES
UNIVERSITY OF HELSINKI

Doctoral Programme in Sustainable Use of Renewable Natural Resources
Doctoral School of Environmental, Food and Biological Sciences
University of Helsinki
Finland

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Siru Salin

ACADEMIC DISSERTATION

To be presented, with the permission of the Faculty of Agriculture and Forestry of the University of Helsinki, for public examination in lecture room B3, Latokartanonkaari 7, Helsinki, on 17 December 2020 at 13.00.

Helsinki, Finland
2020

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Dissertationes Schola Doctoralis Scientiae Circumiectalis, Alimentariae, Biologicae
Publication No. 44/2020

ISBN 978-951-51-6861-0 (Print)
ISBN 978-951-51-6862-7 (Online)
ISSN 2342-5423 (Print)
ISSN 2342-5431 (Online)
Electronic publication at http://ethesis.helsinki.fi

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Cover illustration: Kirsu Moilanen, Castanja Marketing Services Oy, Helsinki
Unigrafia, Helsinki 2020

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ABSTRACT

The research documented in publications I-IV involved studies in dry, late-pregnant Ayrshire dairy cows on grass silage (GS) based diets (I-IV). The principal aim was to investigate the effect of prepartal plasma non-esterified fatty acids (NEFA) level (I) and the effect of prepartal dietary energy intake (II-IV) on the development of insulin resistance (IR) during late pregnancy (I-IV) and changes in insulin resistance in early lactation (II-IV). Detailed, extensive physiological studies were conducted to understand the mechanisms underlying the development of maternal insulin resistance and to investigate the impact of changes in dietary energy level and subsequent changes in plasma NEFA concentration prepartum. The insulin resistance was assessed by interpretation of data from intravenous glucose tolerance test (IVGTT) with minimal model (MM) approach (I-III) and by insulin challenge (IC; I, II) data. Besides insulin resistance, also the impact of prepartal energy intake on metabolic adaptations, tissue deposition and mobilisation as well as dry matter intake (DMI) and lactational performance were investigated in publications III and IV.

In publication I, the key objective was to evaluate the effects of increment of plasma NEFA concentration, typically observed during the last weeks of pregnancy and in early weeks of lactation in dairy cows, on glucose tolerance and responsiveness or sensitivity to insulin as assessed by IVGTT and IC. The greater NEFA levels were achieved by abomasal infusion of tallow (TAL) or camelina oil (CAM). Compared with water infusion (CON), infusion of lipids increased basal plasma NEFA concentrations by around 50%, to an equal level than what was found in dairy cows 2 to 1 weeks prepartum on GS-based diets (II-IV). Elevation of plasma NEFA concentration impaired glucose clearance and decreased insulin secretion during metabolic challenges. These data suggest that elevated plasma NEFA concentrations impaired whole-body insulin responsiveness and sensitivity in dry cows in late pregnancy. As assessed by MM indices, both the disposition and the insulin sensitivity indices were greater after CAM than TAL infusion during IVGTT. Compared with TAL infusion, CAM had an insulin-sensitizing effect which was most likely caused by alterations in plasma profiles of major long-chain fatty acid (FA) groups. A 50% increment in the percentage of polyunsaturated FA (C18:2 and C18:3) and a similar decrease in the percentage of monounsaturated FA (C16:1 and C18:1) was found in plasma FA profiles after CAM infusion when compared with TAL.

In publication II, the dietary effects on insulin resistance were assessed not only by the level of energy intake but also by comparing tissue responses to glucose and insulin in late pregnancy vs. early lactation. Compared with controlled energy intake (CEI), the effect of prepartal overfeeding and gradual restriction of energy (HEI) had a minor effect on whole-body insulin resistance

during the transition period. An attenuated prepartal NEFA response to endogenous insulin was found in HEI cows suggesting a more refractory adipose tissue to insulin than in CEI. After parturition, this effect was reversed. Across the dietary treatments, both basal and stimulated insulin concentration decreased after parturition as a result of a lower response to a similar secretory stimulus than before parturition and due to increased clearance of insulin postpartum. Compared with prepartal IVGTT, glucose disposal was enhanced postpartum across the dietary treatments. A hyperbolic relationship denoted as the disposition index (DI) was observed during the IVGTT. Compared with prepartal glucose and insulin dynamics across the diets, the MM indices point to increased insulin resistance shortly before than shortly after parturition. However, low insulin concentration is the major factor regulating the use of glucose by peripheral tissues in early lactation. The lack of dietary effect on whole-body insulin resistance in publication II was most likely due to minor dietary effect on tissue accretion between treatment groups, although the lower prepartal plasma NEFA concentration in HEI than in CEI cows suggests enhanced lipid deposition in adipose tissue before parturition, facilitated by higher plasma insulin (IV). No dietary effect on plasma hormone and metabolite concentrations or total DMI was found after parturition. High energy intake during the dry period tended to decrease milk yield after calving (IV).

In publication III easily applicable diets suitable for loose housing systems were compared. An ad *libitum* allowance of GS (HEI) induced a more pronounced BW and BCS change prepartum when compared with a GS-diet diluted with wheat straw (CEI). HEI cows demonstrated a compensatory insulin response to glucose in prepartal IVGTT which preserved glucose tolerance of peripheral tissues. The HEI diet reduced and delayed NEFA suppression suggesting decreased insulin sensitivity and responsiveness in adipose tissue prepartum. The high NDF-content in CEI diet probably decreased ruminal propionic acid production as reflected by lower prepartal glucose and insulin CEI cows. Prepartal energy level did not affect metabolic flexibility of transition dairy cows as assessed by the absence of dietary effect on mobilisation of body reserves, plasma metabolites and hormones, and DMI after calving, whereas milk yield was greater from week 5 onward in HEI than in CEI.

The moderate negative effects of gradual restriction of prepartal energy and dilution of energy by mixing GS with wheat straw on early lactation production response demonstrated that these feeding practices were not optimal for transition dairy cows. A moderate or *ad libitum* overfeeding affected peripheral insulin resistance in the level of prepartal lipid metabolism, while *ad libitum* overfeeding of GS induced changes in prepartal glucose metabolism as well. Both the difference in energy intake and the composition of the diet contributed to the observed effects on glucose and NEFA dynamics orchestrated via changes in insulin concentration in the transition period.

Keywords: dairy cow, transition period, grass silage, energy intake, peripheral insulin resistance, adipose tissue lipolysis, plasma hormone and metabolite, milk yield, lipid infusion, plasma NEFA, minimal model

ACKNOWLEDGEMENTS

To my supervisors, docent Tuomo Kokkonen and Prof. Aila Vanhatalo. Tuomo, thank you for the dedicated support and guidance, and most of all, for broadening my scientific thinking and mind, especially in the field of statistics. I am grateful for your patience and for the inspiring conversations that helped me separate the wheat from the chaff. Aila, thank you for your intellectual support throughout this long journey. I greatly appreciate your input especially in the process of finishing the thesis. My appreciation extends to emeritus Prof. Matti Näsi at the University of Helsinki for giving me the opportunity to join this fascinating research project. I owe my warmest appreciation to Dr. Raymond Boston for his enthusiasm and invaluable guidance in the modelling of the metabolic dynamics.

I am also grateful to my co-authors Prof. Juhani Taponen, Docent Kari Elo, Dr. Seija Jaakkola, M.Sc. Ilkka Simpura, for their contributions to this work. Special thanks to Prof. Juhani Taponen for his professional and thorough guidance when teaching me the hands-on skills to perform the metabolic challenges on dairy cows.

I gratefully appreciate the assistance of the staff at the Viikki research farm of Helsinki University for the excellent care of the experimental animals. My warmest thanks extend to technicians Anneli Pakarinen, Leena Luukkainen, Anne Hannikainen, Maija Reijonen and Anna-Liisa Salminen at the laboratory of the Department of Agricultural Sciences for their assistance and guidance when conducting the animal experiments and the chemical and biological analysis.

I want to express my warmest thanks to my fellow doctoral students Dr. Anni Halmemies-Beauchet-Filleau, Dr. Shaimaa Selim, Dr. Rashid Safari, Dr. Nanbing Qin, Dr. Marjukka Lamminen, Dr. Anne Honkanen, Dr. Walter König, Vappu Ylinen, and Laura Kittilä for their support and spirit during these years. My appreciation extends to M.Sc. Milla Frantzi, M.Sc. Hilkka Niemi, M.Sc. Heli Toivonen, M.Sc. Jarno Järvenoja, and M.Sc. Anne Pyhälammi at the University of Helsinki for their assistance in conducting the animal experiments.

Financial support from the Raisio Research Group Foundation, the August Johannes and Aino Tiura Agricultural Research Foundation and the University of Helsinki Research Funds is gratefully acknowledged. My special thanks go to Dr. Ilmo Aronen for the motivation and support throughout these years. This work was partially supported by Raisio Feed Ltd., by Finnish Ministry of Agriculture and Forestry and by Finnish Association of Agronomists.

I am forever grateful for the encouragement and motivation from my friends and family. I extend my warmest thanks to my mother. Thank you for your uplifting words and belief, and unconditional and endless support.

LIST OF ORIGINAL PUBLICATIONS

This thesis is based on the following original articles, which are reproduced with the permission of the copyright holders. The publications are subsequently referred to in the text by their Roman numerals:

I **Salin, S., Taponen, J., Elo, K., Simpura, I., Vanhatalo, A., Boston, R. & Kokkonen, T. 2012.** Effects of abomasal infusion of tallow or camelina oil on responses to glucose and insulin in dairy cows during late pregnancy. Journal of Dairy Science 95: 3812–3825.

II **Salin, S., Vanhatalo, A., Elo, K., Taponen, J., Boston, R.C. & Kokkonen, T. 2017.** Effects of dietary energy allowance and decline in dry matter intake during the dry period on responses to glucose and insulin in transition dairy cows. Journal of Dairy Science 100: 5266-5280.

III **Salin, S., Vanhatalo, A., Elo, K., Taponen, J., Jaakkola, S., Boston, R. C. & Kokkonen, T. 2018.** Effects of dry period energy intake and diet composition on insulin resistance, metabolic adaptation, and production responses in transition dairy cows. Journal of Dairy Science 101: 1-20.

IV **Kokkonen., T., Salin, S., Jaakkola, S., Taponen, J., Elo, K. & Vanhatalo, A. 2018.** Effects of dietary energy allowance in grass silage-based diets during the dry period on production responses and utilization of body reserves in dairy cows. Agricultural and Food Science 27: 264-274.

AUTHOR'S CONTRIBUTION

The contributions of all authors to the original publications of this thesis are described below (initials of authors are listed in alphabetical order).

	Publications			
Phase of work	**I**	**II**	**III**	**IV**
Planning the experiment	TK SS JT AV KE	TK SS JT AV KE	TK SS JT AV KE	TK SS JT AV KE
Conducting the experiment	SS TK JT	SS TK JT	SS TK JT SJ	SS TK JT SJ
Laboratory analysis	SS TK IS	SS TK	SS TK	SS TK
Data analysis	SS TK RB	SS TK RB	SS TK RB	SS TK
Drafting the 1st version of the manuscripts	SS	SS	SS	TK SS
Modifying the manuscripts	SS TK JT AV KE RB	SS TK JT AV KE RB	SS TK JT SJ AV KE RB	TK SS JT SJ AV KE

AV = Aila Vanhatalo
IS = Ilkka Simpura
JT = Juhani Taponen
KE = Kari Elo
RB = Raymond Boston
SJ = Seija Jaakkola
SS = Siru Salin
TK = Tuomo Kokkonen

ABBREVIATIONS

AIRg	acute insulin response
AUC	area under the response curve
BCS	body condition score
BHB	β-hydroxybutyric acid
BW	body weight
CAM	camelina oil
CEI	controlled energy intake
CUDP	close-up dry period
DAG	diacylglycerol
DI	disposition index
EB	energy balance
EI	energy intake
DIM	days in milk
DM	dry matter
DMI	dry matter intake
ECM	energy corrected milk
EXP	experiment
FA	fatty acids
FODP	far-off dry period
GS	grass silage
HEI	high energy intake
IC	insulin challenge
IVGTT	intravenous glucose tolerance test
MAG	monoacylglycerol
ME	metabolizable energy
MER	metabolizable energy requirements
MM	minimal model
MS	maize silage
NDF	neutral detergent fibre
NEFA	non-esterified fatty acids
NSC	non-structural carbohydrates
OM	organic matter
PUFA	polyunsaturated fatty acids
SI	insulin sensitivity index
SFA	saturated fatty acids
TAL	tallow
TMR	total mixed ration
VFA	volatile fatty acids

1 INTRODUCTION

The development of maternal insulin resistance is one of the most important metabolic adaptations in response to altered fuel preference of peripheral tissues with advancing pregnancy and the onset of lactation. This adaptation mechanism ensures that glucose, the vital energy substrate, is continuously and effectively directed to the tissues most in need, namely to the growing fetus and fetal membranes and to the mammary gland shortly before and during early lactation (Bauman and Currie, 1980; Bell, 1995). The transition period extends from three weeks before to three weeks after calving in dairy cows. During this period, insulin-dependent peripheral tissues become less sensitive to the action of insulin, and peripheral insulin resistance develops.

An array of metabolic and homeorhetic changes mediate the development of maternal insulin resistance present in all mammalian species during late pregnancy. In dairy cows, the insulin resistance is suggested to be most pronounced in the transition period (Bell and Bauman, 1997). Subsequent with insulin resistance, the fuel supply in the form of glucose is reduced in insulin-dependent tissues, while hepatic insulin resistance increases gluconeogenesis and glycogenolysis due to deteriorated suppression of these processes by insulin (Brockman and Laarveld, 1986; Hayirli, 2006).

Mobilisation of body reserves from skeletal muscle and adipose tissue initiates shortly before parturition to compensate for the gap between glucose demand and supply. The mobilisation is facilitated by suppression of the inhibitory effect of insulin on lipolysis and by the stimulatory effect on lipogenesis in adipose tissue (Brockman and Laarveld, 1986). Higher plasma NEFA concentration is a valid indicator of increased adipose tissue lipolysis in ruminants, and also reflects decreased insulin sensitivity of adipose tissue, while plasma 3-MH reflects muscle protein breakdown (Pullen et al., 1989; Van der Drift et al., 2012). The NEFA are used as an alternative energy source in peripheral tissues. However, any exaggeration of insulin resistance in adipose tissue, caused for instance by a response to prolonged overfeeding of energy inducing obesity, may further enhance mobilization of NEFA causing additional insulin resistance as part of a vicious cycle (Rosen and Spiegelman, 2006). The increased adipose tissue mass and reduced insulin-mediated suppression of lipolysis associated with i.e. obesity may lead to lipid overflow in the circulation (Jocken and Blaak, 2008). The increased NEFA release associated with prepartal overconditioning may decrease DMI in late pregnancy and decelerate the rate of increase in DMI in early lactation (Grummer et al., 2004). The blood NEFA concentrations typically start to increase 3 to 1 week before parturition, and peak at calving or shortly after parturition to support the energy-deficient state of early lactation (Grummer, 1993; Bertics et al., 1992; Grum et al., 1996; Nielsen et al., 2010). Also limiting the energy intake during the dry period increases NEFA levels and this

increase is more pronounced in overconditioned than in lean cows (Kokkonen et al., 2005; Roche et al., 2015).

The increment of imbalance between energy demand and energy supply (i.e. negative energy balance (EB) during the periparturient period increases NEFA levels due to increased lipid mobilisation (Bauman and Currie, 1980). Consequently, the increased circulating NEFA may predispose the animals to a range of metabolic disorders such as subclinical and clinical ketosis (Grummer et al., 2004; Ospina et al., 2010; McArt et al., 2013) and to hepatic steatosis (Drackley, 1999; Overton and Waldron, 2004). Metabolic health issues are more pronounced in the transition period and decrease the welfare and health of the cows. Subsequently, the decreased production potential increases the economic losses of the farmer (Wallace et al., 1996; Grummer et al., 2004; Drackley et al., 2005).

Hyperinsulinemia is a compensatory mechanism for the deterioration of insulin sensitivity, a phenomenon that precedes impaired glucose tolerance and insulin resistance in humans (Ahren and Pacini, 2004; Bergman, 2007). Also, environmental changes in insulin sensitivity, for instance in response to overfeeding of energy causing obesity, will be compensated by an increase in insulin secretion in response to glucose, as reflected by increased basal insulin concentration (Bergman, 1989; Kahn et al., 1993).

As a result of prepartal overfeeding, the adipose tissue may be more refractory to the actions of insulin. Cows prone to lose great amounts of BW in the transition period were more resistant to insulin's effect on lipolysis inhibition than cows with less BW loss (Zachut et al., 2013). Recently, only minor effects of increased body fatness and high energy intake on inhibition of lipolysis by insulin and insulin signalling in adipose tissue of transition cows have been reported (De Koster et al., 2015, 2016b; Marett et al., 2015; Mann et al., 2016b, Jaakson et al., 2018). As opposed, the overconditioned cows were more insulin resistant in regard to glucose metabolism than leaner cows in late pregnancy (De Koster et al., 2015; Bogaert et al., 2018; Jaakson et al., 2018). Particularly, the increased accretion of adipose tissue depots intensified insulin resistance as assessed by compensated insulin response prepartum (Bogaert et al., 2018; Jaakson et al., 2018). Despite the increased insulin secretion in response to glucose bolus, plasma glucose remained higher during metabolic challenges in overconditioned cows (Bogaert et al., 2018; Jaakson et al., 2018). Increased insulin resistance and refractory glucose metabolism were attributed to pancreatic lipid infiltration and islet hyperplasia (Bogaert et al., 2018) and reduced glucose transport protein synthesis in the adipose tissue (Jaakson et al., 2018).

Impact of insulin resistance in transition dairy cows

Insulin resistance is denoted as a state in which a physiological level of insulin produces a less than normal biological response (Kahn et al., 1978). Insulin resistance is evidenced by alterations in secretion or extraction of insulin, or as a failure of insulin-sensitive tissues to respond to insulin, or both

(Kahn et al., 1978; Sano et al., 1991; Bergman, 2002; Kahn et al., 2006). This homeorhetic adaptation ensures that the most important energy source (glucose) regarding fetal and mammary tissue development is redirected efficiently and continuously to tissues most in need of this energy substrate (Bell, 1995; Bauman, 2000). The glucose uptake into fetus and mammary gland is an insulin-independent process (Laarveld et al., 1981; Faulkner and Pollock, 1990; Reynolds et al., 2003). The so-called "glucose sparing effect of pregnancy" includes changes in the sensitivity and responsiveness to insulin-dependent peripheral tissues (Kahn, 1978; Bell, 1995; De Koster and Opsomer, 2013).

During insulin resistance, in skeletal muscle tissue, which by virtue of its mass is the largest glucose using tissue (Pethick et al., 1984; Bell, 1995; Vernon, 2005; De Koster and Opsomer, 2013), insulin-dependent glucose uptake is decreased (Bauman and Elliot, 1983; Petterson et al., 1993). In ruminants, the total glucose use by the muscles is proportionally greater (40-50%) than that in humans (10-15%), as the human brain and liver have a higher demand for glucose (Hocquette et al., 1996). Further, in ruminants the contribution of adipose tissue to total glucose uptake is minor because rumen-derived lipogenic volatile fatty acid (VFA) acetate is the main precursor for lipogenesis in adipose tissue (Brockman and Laarveld, 1986). As opposed to the former, in monogastric species, glucose is the principal precursor for lipogenesis (Pethick et al., 1984). Glucose uptake occurs by insulin-mediated glucose uptake in insulin-sensitive tissue and by noninsulin-mediated glucose uptake in both insulin-sensitive and insulin-insensitive tissues. The basal glucose transport into the peripheral tissues is mediated by insulin-independent GLUT1 and GLUT 3 transporters (Zhao et al., 1996; Duehlmeier et al., 2005). The insulin-mediated glucose uptake in skeletal muscle, cardiac muscle and adipose tissue is facilitated by type 4 glucose transporter (GLUT4). Insulin stimulates the translocation of GLUT4 to the cell membrane increasing glucose transport activity (Zhou et al., 1999). The rate-limiting step in glucose utilisation in bovine adipose tissue and muscle is glucose transport rate (Hocquette et al., 1996).

In dairy cows, the dependency on gluconeogenesis is accentuated during the transition from pregnancy to lactation. The estimated demand for energy of a Holstein dairy cow to produce 30 kg of milk at 4 days after parturition is tripled relative to the needs of the gravid uterus in late pregnancy. Similarly, the requirements of the mammary gland for fatty acids and amino acids are 2.8, and 4.5 times those of the gravid uterus, respectively (Bell, 1995). Consequently, the basal plasma glucose disappearance in lactating animals is 2 – 4 times greater than that of the dry cow (depending on the production level) comprising to around 3 kg of glucose in a cow producing 40 kg of milk (Bell, 1995; De Koster and Opsomer 2013). Of this glucose, 60 to 90% is preserved for lactose production by the mammary gland (Rose et al., 1997; Bauman and Currie, 1980; De Koster and Opsomer, 2013). The estimated proportion of glucose uptake by insulin-dependent tissues in dry vs. lactating

ruminants are approximately 20% vs. 8%, respectively (Bauman and Currie, 1980; De Koster and Opsomer, 2013)

During the progression of peripheral insulin resistance, maternal tissues must rely on other energy sources that are more readily available. The compensation for decreased fuel supply in skeletal muscle is mediated by alterations in adipose tissue sensitivity to insulin (Bauman and Currie, 1980; Bell, 1995). The sensitivity to insulin is altered for enhanced NEFA release from adipose tissue (Rukkwamsuk et al., 1998; Nielsen et al., 2010), reflected by changes in circulating NEFA concentrations (Pullen et al., 1989; Guo et al., 2007). The naturally occurring decrement of basal insulin level shortly before parturition supports the lipolytic stage of the body and enhances hepatic gluconeogenesis to cope with the energy deficit (Bauman and Currie, 1980; Bell, 1995; Ingvartsen and Andersen, 2000). It has been suggested that the decrease of blood insulin concentration is more a result of decreased pancreatic output than that of increased hepatic extraction in dairy cows (Reynolds et al., 2003). Indeed, very early work with dairy cows showed that pancreatic responsiveness to insulinotropic agents is dramatically decreased after parturition (Lomax et al., 1979), and subsequent research has verified the decrement of insulin secretion in early lactation (Holtenius et al., 2003; Bossaert et al., 2008; Weber et al., 2016). In overconditioned cows, greater prepartal insulin concentrations in response to glucose bolus were reported recently (Bogaert et al., 2018; Jaakson et al., 2018) while higher postpartal loss of body weight and prepartal overconditioning induced lower insulin levels postpartum (Zachut et al., 2013, Mann et al., 2016a).

However, even among lean subjects with normal glucose tolerance, there is a large variation between individuals in insulin sensitivity (Hollenbeck and Reaven, 1978), suggesting that also other factors, such as genetics, may determine sensitivity under baseline conditions in humans (Watanabe, 2010; Ader et al., 2014). Similarly, variability in response to altered fuel preferences in dairy cows at the onset of lactation has been attributed to genetic factors (Bossaert et al., 2008; Kessel et al., 2008). Also strain differences in basal and glucose-stimulated insulin secretion as well as in insulin responsiveness to glucose bolus have been reported in dairy cows (Shingu et al., 2002; Bossert et al., 2008; Chagas et al., 2009). The selection for greater milk yield potential is reportedly associated with lower basal insulin concentrations (Veerkamp and Koenen, 2006; Bossaert et al., 2009) and to lower glucose-stimulated insulin response (Hammon et al., 2009).

The insulin resistance of the transition period in dairy cows has gained considerable interest during the last years. Reported findings of studies investigating insulin resistance by different assessment methods have not reached a consensus on whether maternal insulin resistance is sustained after parturition in dairy cows, or not. The historical studies conducted both in small and large ruminants suggested that insulin resistance is more pronounced in early lactation than in late pregnancy (Lomax et al., 1979; Vernon et al., 1990; Debras et al., 1989; Bell, 1995; Bell and Bauman, 1997).

The putative postpartal increment of insulin resistance is associated with a decreased maximal insulin response to glucose (Lomax et al., 1979; Bell 1995) and reduced glucose uptake by insulin-sensitive tissues (Vernon et al., 1990). Studies showing that glucose is spared for non-insulin-dependent fetal membranes in late pregnancy and the mammary gland in early lactation (Bauman and Currie, 1980; Etherton and Bauman, 1988; Bell, 1995) reinforced the hypothesis that insulin resistance extends to early lactation in ruminant species. However, newer studies (Smith et al., 2004; Marett et al., 2015; Mann et al., 2016a; Weber et al., 2016) showed minor changes in insulin responsiveness and sensitivity of various tissues between early lactation and late pregnancy. Also, repeated metabolic challenges during extended lactation indicated negligible effects of energy level on peripheral insulin resistance (Marett et al., 2015), while indications of improvement in the overall sensitivity of tissues to insulin in early lactation have been published as well (Kräft et al., 2004; Stanley, 2005; Oliveira et al., 2016). Discrepancies between studies may arise from a range of factors, such as differences in the timing of the challenges relative to parturition, in feed composition and DMI and thus, in production level, in breed and strain, and in body condition of the cow.

Additional aspects of metabolic and hormonal adaptation

The physiological adaptation of transition from late pregnancy to early lactation period includes not only alterations in tissue sensitivity to circulating insulin but also a range of other endocrine changes. These are reflected by adjustments in circulating levels of metabolites and hormone concentrations. Around calving, cows enter a state of negative EB during which they are unable to consume enough feed to support the demands of both lactation and maintenance (Bauman and Currie, 1980; Bauman, 2000). This paradoxical incidence where DMI lags behind the copious milk production is reflected by the metabolic profile. The metabolic milieu of early lactation dairy cow is characterized by a decrement of circulating concentrations of insulin and glucose and increment of concentrations of NEFA and BHB. Glucagon concentrations are upregulated at the onset of lactation as well (Vazquez-Añon et al., 1994; De Koster and Opsomer, 2013) and contribute to increased oxidation of NEFA, and adjustments in plasma glucose concentrations mainly by enhancing hepatic gluconeogenesis (Bobe et al., 2003; Aschenbach et al., 2010).

When cows enter negative EB in early lactation, plasma growth hormone (GH) facilitates NEFA flux from adipose tissue by increasing lipolytic response to β-adrenergic signals and by attenuating insulin-mediated lipogenesis and glucose utilization in the peripheral tissues (Bauman and Vernon, 1993; Etherton and Bauman, 1998). Also, changes in hormone concentrations associated with reproduction (estradiol, progesterone and prolactin) are known to modify adipose tissue responses both to insulin and adrenergic stimuli (Bell, 1995; McNamara, 1997; Hayirli, 2006). Additionally, catecholamines and glucocorticoids increase around calving (Smith et al.,

1973; Chilliard et al., 2000; Drackley et al., 2005). These regulatory mechanisms are directed to supply the mammary gland with essential precursors for milk synthesis (Bell, 1995; Bauman, 2000) and simultaneously diminishing the use of glucose in peripheral tissues. The range of complex adaptations occurs gradually during the transition period and vary considerably between individuals (Jorritsma et al., 2003; Kessel et al., 2008; Weber et al., 2013). Considering that the production potential of the modern dairy cow has increased dramatically over the past decades, it is vital to sustain the metabolic flexibility of the transition dairy cow by supporting the rapid changes in metabolism with optimal nutrition during this vulnerable period of the production cycle. Metabolic flexibility is stated to represent the ability to adjust fuel oxidation to fuel availability (i.e. from basal to stimulated conditions; Kelley and Mandarino, 2000).

During the last two decades, research has reached consensus in showing that metabolizable energy (ME) intake of dry cows should be controlled to the level of requirements at least in cows on maize-silage (MS) based diets (Drackley et al., 2005; Dann et al., 2006; Janovick et al., 2011; Cardoso et al., 2013). Overfeeding of energy, particularly with grains providing higher non-fiber-carbohydrates (NFC) in the diets, cause hyperglycemia and hyperinsulinemia already prepartum. (Holtenius et al., 2003; Dann et al., 2006; Douglas et al., 2006; Janovick et al., 2011).

Energy intake can be controlled either by restricting the amount of feed offered or by modifications of the diet composition. Several recent studies (e.g. Dann et al., 2006; Janovick et al., 2011; Litherland et al., 2012; Mann et al., 2015) have evaluated the use of wheat straw in the diets of dry cows containing maize silage (MS). Only limited published research has evaluated controlling energy content of GS-based diets, by using low-energy forage sources, such as secondary regrowth GS (Litherland et al., 2013; Little et al., 2016) or straw (Dewhurst et al., 2000; Agenäs et al., 2003; McNamara et al., 2003). Accordingly, the research on the effect of GS allowance as sole feed in dairy cows during the far-off dry period (FODP) is limited to few studies (Dewhurst et al., 2009; Little et al., 2016). The effect of decreasing the oversupply of energy intake during the close-up dry period (CUDP) has not been studied experimentally on GS, although large changes in prepartal DMI have been linked to lower postpartal DMI (Grummer et al., 2004; Drackley et al., 2005). Indeed, feeding to meet or underscore the energy requirements, as compared to *ad libitum* feeding during the FODP resulted in lower circulating postpartal NEFA (Dann et al., 2006; Cardoso et al., 2013) with a positive effect on DMI and energy intake during the first weeks of lactation (Dann et al., 2006; Cardoso et al., 2013).

Assessment of insulin sensitivity in dairy cows

Hyperinsulinemic-euglycemic clamp technique (HEC) is the gold standard method for the assessment of insulin sensitivity in humans and animals. Intravenous glucose tolerance test (IVGTT) has been used in several species,

including bovine, and it is one of the most accurate methods for the assessment of exogenous glucose response in peripheral tissues in animals in normal physiological conditions. However, given the extraordinary glucose metabolism of the ruminants (reviewed e.g. by Aschenbach et al., 2010; De Koster and Opsomer, 2013), it has been argued that IVGTT performed in dairy cows during the transition period may be confounded by the massive glucose uptake by the mammary gland occurring insulin-independently (Schoenberg and Overton, 2010; De Koster and Opsomer, 2013; Mann et al., 2016a). As the discrimination between insulin-dependent and insulin-independent glucose uptake during the IVGTT is challenging, additional modelling is preferable in order to increase the interpretability of the values derived from standard non-insulin modified IVGTT. The MM is widely used in human and animal studies and it is one of the most accurate ways to assess the reciprocal regulation of insulin and glucose during the IVGTT (Bergman et al., 1987; Ferrannini and Mari, 1998; Muniyappa et al., 2008; Ader et al., 2014). Some studies have applied IVGTT with the MM for the investigation of insulin resistance in dairy cows (Stanley, 2005; Moate et al., 2007; Marett et al., 2015; De Koster et al., 2016a, 2017; Bogaert et al., 2018). Also, other surrogate indices and additional calculated values describing metabolic dynamics have been used in dairy cattle (e.g. Holtenius and Holtenius, 2007; Bossaert et al., 2009; Kerestes et al., 2009; Schoenberg et al., 2012; De Koster et al., 2016; Mann et al., 2016a; Weber et al., 2016). A NEFA model (Boston and Moate, 2008) may also be incorporated into IVGTT data to assess the effect of insulin on adipose tissue lipolysis inhibition.

During the non-insulin modified IVGTT, the development of peripheral insulin resistance can be observed by alterations in both NEFA and glucose dynamics and insulin response to an exogenous glucose load. The deterioration of glucose uptake by insulin-sensitive tissues is evidenced as larger AUC of glucose, slower removal of glucose (lower clearance rate of glucose; CR), higher glucose half-life (T1/2), or all of these, providing that insulin secretion is not altered. Differences in peak glucose concentrations during an IVGTT are discussed as being implicative of changes in tissue insulin responsiveness (Kahn, 1978; Schoenberg et al., 2012), providing that insulin concentrations are similar between evaluated treatment groups during the IVGTT (Hayirli et al., 2001; Hayirli, 2006). Glucose concentrations during IVGTT depend on glucose consumption by peripheral tissues, endogenous glucose production mainly by the liver, renal glucose excretion, and intestinal glucose absorption (Pires et al., 2008).

Recently, there have also been attempts to study the associations of negative EB of early lactation and insulin resistance via induction of greater NEFA levels. The hyperlipidemia has been induced by feed restriction or by lipid infusion, or both to mimic the energy-deficient state. Also, agents that inhibit lipolysis have been used in dry dairy cows to assess the effect of induced higher NEFA on insulin resistance (Pires et al., 2007b, 2008; Schoenberg and Overton 2011; Schoenberg et al., 2012). Higher plasma NEFA are reportedly

associated with the induction of insulin resistance in dry non-pregnant and pregnant cows (Pires et al., 2007b; Schoenberg et al., 2012).

2 OBJECTIVES AND HYPOTHESES

The ultimate objective of the three experiments presented in this thesis was to investigate the metabolic dynamics underlying the state of insulin resistance of dairy cows transitioning from pregnancy to early lactation. The primary target was to study the associations between higher vs. controlled energy intake during the dry period and the development of maternal insulin resistance in pregnant and early lactation in dairy cows. The emphasis was on the investigation of insulin resistance of the transition period by means of IVGTT with MM approach, and by insulin challenges (IC).

In experiment 1 (I) abomasal infusions of tallow (TAL) and camelina oil (CAM) were used as a method to induce higher plasma NEFA levels in dry, late pregnant cows in order to study the effect of elevated circulating NEFA and alterations in plasma fatty acid concentrations on insulin resistance. This experimental setup served as a model in studying the effects of higher NEFA levels observed typically near calving. Subsequent experiments evaluated the effect of high vs. controlled energy intake during the dry period (II, III, IV) on peripheral insulin resistance (II, III) and accretion and mobilisation of body reserves and energy balance (III, IV), and dry matter intake (DMI) and lactation performance (III, IV). The experiments aimed to study easily applicable feeding practices to loose-housing systems during the indoor feeding period in the Northern countries on typical GS- based diets. Controlled or limited energy allowance was implemented by restricting GS allowance (II, IV) or by dilution of GS-diet with wheat straw to allow for physical limitation of DMI to occur (III).

The hypotheses tested in this research were:

• Abomasal infusion of lipids increase plasma NEFA concentration in dry cows and higher NEFA levels induce a more pronounced insulin resistance of peripheral tissues.

• Abomasal infusion of tallow increases the proportion of saturated fatty acids (SFA) in plasma lipids and deteriorate the maternal insulin resistance in late pregnant dry dairy cows as opposed to an insulin sensitizing effect of abomasal infusion of camelina oil to increase the concentration of plasma polyunsaturated fatty acids (PUFA) (I).

• Overfeeding of moderately digestible GS during the dry period (II, III) increases maternal insulin resistance prior to calving when compared with controlled (II) or limited energy intake (III), and that this effect carries over to the early lactation (II, III).

• Overfeeding of moderately digestible GS during the dry period (III, IV) increases lipid accretion in late pregnancy, and adipose tissue mobilisation after parturition decelerating the increase in DMI in early lactation (III, IV) when compared with controlled (II) or limited energy intake (III).

3 SUMMARY OF MATERIALS AND METHODS

3.1 EXPERIMENTAL DESIGN AND ANIMALS

The studies discussed in this thesis and documented in publications I to IV were conducted as three experiments (Exp. 1 – 3). The experiments are described in more details in the original publications, here a rough outline of the studies is described (Table 1).

The first Exp. 1 (I) involved dry, late-pregnant, second-parity, rumen-cannulated Finnish Ayrshire dairy cows (n = 6). The animals were dried off 73 ± 4 d (mean ± SD) before their expected parturition date, housed in tie-stalls, and offered GS *ad libitum* with a mineral mixture until the initiation of the experiment. Cows were randomly assigned to treatments in a replicated 3 × 3 Latin square design 45 ± 3 d and 43 ± 4 d (mean ± SD) before the expected and actual dates of calving, respectively. The length of each experimental period was 5 d. To reduce carry-over effects, a 5-d washout period followed each experimental period. In the end of the experiment, cows averaged 19 ± 4 d (mean ± SD) apart from their due dates.

In the two consecutive experiments (Exp. 2 and 3), multiparous, dry, Finnish Ayrshire dairy cows were used (n = 16 in each) ranging from 2nd to 5th lactation. The cows were dried off either before or on the day of the initiation of the experimental period (Exp. 2 and 3). The milk yield of cows averaged 10500 kg and 10300 ± 1500 kg (mean ± SD) from the period preceding lactation in Exp. 2 and 3, respectively.

3.2 EXPERIMENTAL TREATMENTS AND DIETS

The first Exp. 1 (I), was designed to investigate the effects of experimental elevation of plasma NEFA concentration by abomasal infusions of TAL or CAM on whole-body responses to exogenous glucose and insulin when compared with CON infusion. By abomasal infusions of lipids, the aim was to achieve similar levels of basal plasma NEFA concentrations than those typically reported in dairy cows on GS-based diets towards the end of the dry period (Holtenius et al., 2003; Kokkonen et al., 2004, 2005). It was assessed whether CAM, rich in C18:2 and alpha linoleic acid C18:3n-3 (37% of total FA) enhances whole-body insulin sensitivity compared with TAL, the FA profile of which resembles that of a cow's fat depots (Smith et al., 1978). In TAL infusate, the three quantitatively most abundant fatty acids present in ruminant adipose tissue accounted for 87.6% of total FA; the saturated palmitic acid (C16:0; 27.2 % of total FA) and stearic acid (18:0; 23.5 % of total FA), and the monounsaturated oleic acid (18:1; 36.9 % of total FA). Cows were fed a mixture

of GS and grass hay (80%:20% of DM) to meet 95% of the ME requirements of 8-mo-pregnant cows (Luke, 2019), and the energy content of the lipid infusions was taken into account in the calculation of individual energy allowances. During the 5 d washout periods, cows were given the same forage mixture to meet 100% of ME requirements.

In Exp. 2 (II, IV), the effect of different energy allowances during the dry period on insulin resistance (II) and on DMI, metabolic profiles, body composition, mobilisation and lactation performance in dairy cows on GS was investigated (IV). The combined effect of a HEI during the FODP and the subsequent decline in DMI with approaching calving observed previously in abundantly fed overconditioned cows (Grummer et al., 2004; Dann et al., 2006; Douglas et al., 2006) was studied, while the daily ration of the control-fed cows (CEI) was limited to 100% of MER (Luke, 2019). Both groups were fed wilted GS during the first 3 wk of the dry period (FODP) and wilted GS supplemented with a commercial concentrate (30% of calculated ME intake/d) during the final 3 wk of pregnancy (CUDP). In the case of postdate pregnancies, the same amount of feed was offered until the due date. Postpartum, a similar lactation diet with *ad libitum* access to GS and increasing concentrate allowance (max. 16 kg/d at 32 d of lactation) was fed for all cows.

The Exp. 3 (III) was designed to be an easily applicable feeding strategy to loose-housing systems on GS-based diets. The study compared the effects of *ad libitum* allowance of GS (HEI) with a GS-based total-mixed ration (CEI) during the whole 8 wk dry period on whole body insulin sensitivity, metabolic adaptations and lactation performance (III). The TMR consisted of GS, wheat straw and rapeseed meal (55/40/5%). Commercial concentrates were fed 1 and 2 kg/d during the last 10–6 and 5–0 d before the expected calving date, respectively. Postpartum, a similar lactation diet with *ad libitum* access to GS and increasing concentrate allowance (max. 16 kg/d at 32 d of lactation) was fed for all cows.

3.3 MEASUREMENTS AND EXPERIMENTAL PROCEDURES

All experimental procedures were conducted under the protocols approved by the National Animal Ethics Committee in Finland in accordance with guidelines established by the European Community Council Directive 86/609/EEC. The amount of feed offered and that refused was recorded daily for the determination of feed intake (Exp. 1 – 3). The feeds were sampled weekly, and the concentrate and silage samples were pooled to form a weekly (Exp. 1) or monthly sample (Exp. 2 and 3). During each experimental period (Exp. 1), faecal samples were collected from the rectum twice daily and pooled on an

Table 1. Summary of experiments documented in publications

Publication (Exp.)	Design and animals	Treatments and diets	ME intake (% of MER)	Measurements	Effects on
I (1)	2 x 3 Latin square	Abomasal infusion of		IVGTT	Plasma
	replicated	• water (CON)	95%	• minimal model	• glucose
	-40 to -20 d[1]	• tallow (TAL)	95%	• NEFA model	• insulin
	5 d + 5 d	• camelina oil (CAM)	95%	IC	• NEFA
	(TRT + flushing) period	basal diet:		DMI	Plasma FA profiles
	6 rumen-cannulated	GS/GH (80%/20%)		Blood Samples	IR
	2nd parity AYDC			Feed samples	EB, DMI, apparent digestibility
II, IV (2)	RCB design	Overfeeding (HEI) vs.	CEI HEI	IVGTT	Plasma
	- 6 to + 8 wk[1]	Controlled (CEI)		• minimal model	• glucose
	16 multiparous	allowance of diets:		• NEFA model	• insulin
	pregnant AYDC	FODP (-6 to -4 wk[1])	100% 144%	IC	• NEFA
		CUDP (-3 to 0 wk[1])	100% 119%	DMI	• glucagon
		Diets (HEI & CEI):		BW	• BHB
		FODP: GS		BCS	• glycerol
		CUDP: GS + concentrates (30% of		Blood samples	• 3-MH
		of ME/d)		Feed & milk samples	IR, BCS, BW, EB, DMI, milk yield
III (3)	RCB design	Overfeeding (HEI) vs.	CEI HEI	IVGTT	Plasma
	- 8 to + 8 wk[1]	Controlled (CEI)		• minimal model	• glucose
	16 multiparous	allowance of diets:	108% 141%	• NEFA model	• insulin
	pregnant AYDC	HEI:		DMI	• NEFA
		ad lib allowance of GS		BW	• glucagon
		-10 – 0 d[1]; concentrates 1 - 2 kg		BCS	• BHB
		CEI diet:		Blood samples	• glycerol
		GS, WS, RSM		Feed & milk samples	• 3-MH
		55%, 45%, 5%		Back muscle diameter	
		-10 – 0 d[1]; concentrates 1 - 2 kg			IR, BCS, BW, EB, DMI, milk yield

[1] Weeks (wk) and days (d) to expected parturition;

Exp. = experiment; ME = metabolizable energy; MER = ME requirements (MJ(d); TRT = treatment; AYDC = Ayrshire dairy cow; GS= grass silage; GH = grass hay; IVGTT = intravenous glucose tolerance test; IC = insulin challenge; DMI = dry matter intake; NEFA = non-esterified fatty acids; FA = fatty acids; IR = insulin resistance; EB = energy balance; RCB = randomized complete block; FODP = Far-off dry period; CUDP = close-up dry period; BW = body weight; BCS = Body condition score; BHB = β-hydroxybutyric acid; 3-MH = 3-metylhistidine; WS = wheat straw; RSM = rapeseed meal.

individual cow basis at the end of each period. Diet digestibility was measured by using acid insoluble ash as a marker (Exp. 1). Feed samples were analysed as described in each publication.

Daily milk yields were recorded in each milking after parturition and samples for the milk composition analysis were collected (Exp. 2 and 3) on four consecutive milkings, and composited according to yield at 1, 2, 4, 6 (Exp. 2 and 3) and 8 (Exp. 3) wk after parturition.

During the first 14 d (Exp. 2) and 10 d (Exp. 3) of lactation, the cows were kept in tie stalls and milked twice daily at 0630 and 1700 h. After this period until 8 wk postpartum, the cows were moved to a free-stall barn equipped with automated roughage feeding troughs and concentrate feeders and milked with an automated milking system (Exp. 2 and 3).

Cows were weighed on two consecutive days before the initiation of every experimental period, and BCS was recorded at the beginning and end of the experiment (Exp. 1 – 3). Body weights, were recorded at the same time of day, on two consecutive days, and BCS at 8 (Exp. 3) 6, 4, 2 and 1 wk (Exp. 2 and 3) before the expected calving date, on the day of calving, the following day, and at 1, 2, 4, 6 (Exp. 2 and 3) and 8 wk after calving (Exp. 3). The cross section of the longissimus dorsi muscle (pars lumbalis) and the subcutaneous adipose tissue thickness were measured on the right transversal process of the third lumbar vertebra, 2 to 3 cm medially from the lateral end at 14 days prior to, and at 1, 7 and 28 d after parturition (Exp. 3).

Basal weekly blood samples were drawn from coccygeal vessels at 56 (Exp. 3), 42, 28, 21, 16, 12, 7, 5, 3 and 1 d (Exp. 2 and 3) before the expected calving date and at 1, 3, 5, 7, 14, 21, 28, 42 (Exp. 2 and 3) and 56 d (Exp. 3) after calving for analyses of glucose, insulin, glucagon, β-hydroxybutyrate (BHB), NEFA, and glycerol. Plasma samples for 3-methylhistidine (3-MH) were collected at 12 days prior to the expected calving, and at 1, 7 and 28 d after parturition (Exp. 2 and 3). All samples were stored and handled as described in the publications (I-IV).

3.3.1 ABOMASAL INFUSION OF LIPIDS

In Exp. 1, an amount of 500 mL/d (approximately 430 g/d of TAL or CAM) of water or lipid was infused through an abomasal line attached to the rumen cannula plug. The infusion line (polyvinyl chloride tubing, i.d. 6.0 mm) was anchored in the abomasum with a sinker (polyethylene bottles filled with ball bearings, approximately 450 g) attached to the distal end of the line. Placement of the infusion line was checked twice daily by hand. The treatments were administered in 10 equal portions (50 mL each) every second hour between 0600 and 2400 h with a 100-mL syringe. For the tallow infusion, the daily amount of tallow was melted in a convection oven at 50°C for 12 h from the previous evening onwards and stored at the same temperature during the day of infusion. To avoid solidification of tallow, the lipid- and water-filled syringes were kept in a water bath (44°C) until lipid

supplements were infused into the abomasum. Boiled tap water (150 mL; 37°C) and ethanol (10 mL) were infused simultaneously with the control treatments to flush the abomasal lines. The liquids were infused in following sequence: 50 mL of water, 50 mL of lipid (water in control treatment), 50 mL of water, 10 mL of ethanol, and 50 mL of water. Treatments were administered for 98 and 108 h before IVGTT and IC, respectively (I).

3.3.2 INTRAVENOUS GLUCOSE TOLERANCE TEST (IVGTT)

A non-insulin modified frequently sampled IVGTT was conducted in Exp. 1 (I) and 2 (II) by collecting basal plasma samples at −15, −5, 5, 10, 15, 20, 30, 40, 50, 60, 90, 120, 150, and 180 min relative to the initiation of glucose infusion. In experiment 3 (III), the cows were subjected to a standard frequently sampled IVGTT to gain more detailed data on metabolite and hormone dynamics during the challenge. Blood samples were collected via catheters at -10, -5, 0, 1, 2, 3, 4, 5, 6, 7, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30, 40, 50, 60, 70, 80, 90, 120, 150, 180, 210 and 240 min relative to the initiation of glucose infusion.

In Exp. 1, IVGTT was performed on d 5 at 0900 h of each experimental period, during which treatment infusions were suspended. In Exp. 2, IVGTT was performed at 0900 h 10 ± 5 d (n = 15) prior to the actual delivery date and 10 ± 1 d (n = 14) postpartum, and in Exp. 3 at 0830 h 13 ± 5 d (n = 16) prior to actual delivery date and 9 ± 1 d (n = 16) postpartum. For the IVGTT, a glucose bolus (250 mg/kg of BW, glucose 300 mg/mL) was administered through a catheter fitted into the left jugular vein over 4.0 ± 1.8 min (mean ± SD) in Exp. 1, and over 6.5 ± 3.5 and 4.2 ± 1.7 min pre- and postpartum in experiment 2, respectively, and over 4.5 ± 1.4 and 4.6 ± 2.3 min pre- and postpartum, in Exp. 3, respectively. Blood samples for the determination of glucose, insulin and NEFA concentrations were obtained from a catheter fitted into the right jugular vein (Exp. 1-3).

Feed but not water was withheld for 1 hour (Exp. 1 and 2), and for 2 hours (Exp. 3) before the initiation of the IVGTT.

3.3.3 INSULIN CHALLENGE (IC)

Intravenous insulin challenges were performed on the same day as IVGTT at 1900 h by i.v. administration of 0.1 IU of insulin/kg of BW (100 IU of insulin/mL of solution; Exp. 1 and 2).

During the challenges, blood samples were collected from the right jugular vein, whereas the injection of insulin for the IC was administered into the left jugular vein. Blood samples were collected at −15, −5, 5, 10, 15, 20, 30, 40, 50, 60, 90, and 120 min relative to administration of insulin.

The treatment infusions were suspended during IC in Exp. 1.

3.4 CALCULATIONS

3.4.1 IVGTT AND IC

Insulin and glucose peak concentrations (I-III) and nadir of NEFA (I-III) were determined from the raw data. To estimate the clearance of metabolites during IVGTT (I-III) and IC (I, II), clearance rate (CR; %/min) and time to reach half-maximal concentration (T1/2; min, I) of metabolites were calculated using PROC NLIN of SAS (versions 9.1 – 9.3). Exponential curves for glucose, insulin, and NEFA concentrations during metabolic challenges were fitted using the following equation (I-III):

(1) $F(t)= A \times e(-k \times t)$,

where F(t) is the metabolite or hormone concentration at time t; A is the estimated maximum value of glucose or basal value of insulin or NEFA for IVGTT, and estimated maximum insulin concentration or basal glucose or NEFA concentration for IC; t is the time (min); and k is the regression coefficient. The following parameters were calculated:

(2) $T½ = (\ln[2]/CR) \times 100$,

(3) $CR = 100 \times (\ln[ta] - \ln[tb])/(tb - ta)$,

where [ta] is the concentration of metabolite at time a (ta), and [tb] is the concentration of metabolite at time b (tb).

Plasma glucose, insulin, and NEFA responses to metabolic challenges were calculated as a net incremental AUC (mmol/L × min for glucose and NEFA; µIU/ mL × min for insulin) during the first 30 (II), 60 and 180 (I-III) and 240 min (III) min of the IVGTT and 30 and 120 min of the IC for NEFA (I, II) and glucose (I, II) and insulin (I) using the actual concentration values. The net incremental AUC was calculated by SAS (version 9.3; SAS Institute Inc., Cary, NC) using the trapezoidal rule (Shiang, 2004) in which basal concentrations were calculated as mean concentrations of the blood samples taken 15 and 5 min (I, II) and 10 and 5 min (III) before the IVGTT and IC (I, II). Insulin, glucose, and NEFA peaks and nadir concentrations were determined. The NEFA decrement was calculated by subtracting the nadir concentrations from the basal concentrations (III).

3.4.2 ESTIMATES OF GLUCOSE USE BY DIFFERENT TISSUES

In Exp. 2 (II) additional calculations on the estimates of glucose use by different peripheral tissues were made by assuming that the net incremental AUC of glucose during 180 min of IVGTT represents (1) total glucose exposure

and (2) complete disposal of infused glucose (i.e., the state when glucose concentration has returned to basal level). Accordingly, AUC180 represents a state when all exogenous glucose is used, and AUC at time t represents a state of partial glucose exposure when AUCt/ AUC180 × 100% of exogenous glucose is used. The glucose requirement of the gravid uterus was assumed to be 0.10 mol/kg of fetus per day, and the glucose requirement of the mammary gland during lactation was 0.4 mol/kg of milk (as summarized by De Koster and Opsomer, 2013). These estimates were used to calculate the glucose requirement (per hour) of the gravid uterus and milk synthesis during IVGTT. Glucose use for milk synthesis was assumed to be constant during IVGTT because no change in the rate of lactose synthesis of isolated bovine mammary cells over the range of 3 to 20 mmol/L of glucose has been reported (Rao et al., 1975). The excretion of glucose in the urine was assumed to be 5% of the infused amount based on observations by Grünberg et al. (2011) showing that 4 to 7% of dextrose was excreted in the urine during the IVGTT using an infusion dose of 0.3 to 0.4 g/kg of BW.

3.4.3 MINIMAL MODEL ANALYSIS

For the IVGTT (I-III), the insulin sensitivity index (SI; $\times 10^{-4}$ min^{-1}/μIU/mL), acute insulin response (AIR_G; μIU/ mL), and disposition index (DI; the product of SI and AIR_G) were obtained by analysing glucose and insulin concentrations of individual animals calculated with the MinMod program (Boston et al., 2003) using the MM (Bergman et al., 1987; Bergman, 1989). The index SI represents the effect of plasma insulin to increase the fractional disappearance rate of glucose; AIR_G is the first-phase insulin response to glucose load; DI reflects the ability of the β-cells of the pancreatic islets to compensate for insulin resistance by increasing β-cell responsiveness, and Sg represents the capacity of glucose *per se* to promote its disposal by peripheral tissues and to suppress endogenous glucose production (Bergman 1989; Best et al., 1996; Bergman, 2002).

The hyperbola describing relationship between MM-derived indices AIRg and SI, namely the DI, was generated from extrapolated values of insulin secretion (AIRg) based on the average of observed values of DI for –10 d and +10 d (II) and for –13 d and +9 d (III). The extrapolation was done by varying SI in the range from 0.0625 to 10 (II) and from 0.01 to 6 (III; Stefanovski et al., 2011).

3.4.4 NEFA MODEL ANALYSIS

For the evaluation of NEFA disposal during IVGTT, a NEFA model (Boston and Moate, 2008) was used to obtain following parameters: FFA0 (μmol/L), the initial plasma NEFA concentration at time zero relative to glucose infusion, S_{FFA} (mmol/L per min) describing the maximal rate of net provision of NEFA to the plasma pool, and K_{FFA} (%/min) describes the rate at which NEFA leaves

the plasma pool. Latency describes the time between glucose challenge and the point when glucose reaches the adipocyte and triggers the suppression of lipolysis (Boston and Moate, 2008).

3.5 STATISTICAL ANALYSES

Prior to statistical analysis, residuals of all data were checked for normality using the MIXED and UNIVARIATE procedures of SAS (I-IV). Logarithmic transformation was used to correct for deviations from normality and homoscedasticity of residuals when needed (I-IV). In cases with non-normal distribution, the untransformed values with P-values from the statistical analysis of the natural logarithmic transformed values are presented (I-II), however, back transformed values are shown in publications III and IV.

Data in Exp. 1 (I) were analysed by ANOVA using the Mixed procedure of SAS (version 9.1, SAS Institute Inc.). The model included fixed effects of treatment, square and period within square and random effects of cow within square. Period was removed from the statistical model when declared nonsignificant at $P > 0.20$. Predefined orthogonal contrasts were used to test the effects of lipid infusions: lipids versus control and CAM versus TAL (I).

In publications III and IV, the pre- and the postpartal data were analysed separately, whereas the IVGTT derived data (–10 d and +10 d relative to calving) in Exp. 2 (II) was combined. The data of BW, BCS, and EB at particular time points and their changes and prepartal plasma 3-MH and back muscle and back fat diameter (III, IV), as well as data derived from IVGTT (III) were analysed by ANOVA with a model including a fixed effect of treatment and a random effect of block using the MIXED procedure of SAS (version 9.3; SAS Institute Inc.). Measurements of DMI, EB, and milk production were reduced to weekly means before statistical analysis (III, IV). The data for feed intake, milk production, blood basal hormone and metabolite concentrations, BW, BCS, EB, plasma 3-MH and back muscle diameter (III, IV) and the pre- and postpartal combined data of IVGTT (II) were analysed as repeated measures ANOVA using the MIXED procedure of SAS. The statistical model included a fixed effect of treatment, time (day or week relative to calving), and the interaction between treatment and time (diet × time) and a random effect of block and interaction between block and time. Where interactions with day or week were significant, the MIXED procedure with slice option in SAS was used to separate treatment by time effects. Degrees of freedom were estimated by using the Kenward-Roger option in the model statement. For each variable analysed, cow nested within the treatment was subjected to 3 covariance structures: compound symmetry (CS), unstructured (UN) and autoregressive order 1 (AR(1)). For unequally spaced measures, spatial power (SP(POW)) was used instead of AR(1). The covariance structure that resulted in the smallest Schwarz Bayesian information criterion was used (Littell et al., 1996).

The relationships between plasma concentrations, parameters describing insulin sensitivity, and the interval between the sampling day and the actual day of parturition were investigated by Spearman's correlation analysis using the CORR procedure of SAS (II, III).

4 RESULTS AND DISCUSSION

4.1 ANIMAL PERFORMANCE

4.1.1 FEED INTAKE AND ENERGY BALANCE PREPARTUM

Effect of induced higher plasma NEFA (I)

To induce higher circulating NEFA levels the cows were fed to meet 95% of ME-requirements (Luke 2010) and infused with lipids (0.43 kg of approximately 100% DM) of which energy content was taken into account in daily MER calculations. The feeding practice led to an average daily DMI of 8.7 kg vs. 7.35 kg/d in water vs. lipid treatments, respectively. The average daily amount of lipid infusions comprised to an average ME intake of 14 MJ/d during the lipid infusions, which corresponds to 1,3 kg less DMI of TMR/d during the lipid infusions. The daily DMI difference indicate that the cows did not decrease the forage intake during the infusion periods (I). In contrast to current results (I), abomasal infusion of lipids have reportedly decreased DMI (Allen, 2000; Rabiee et al., 2012), especially when mainly saturated fatty acids containing lipids, such as TAL, has been added to the rations. A range of proposed mechanisms are involved in the observed decrement in DMI associated with supplemental fat in ruminants; chemical and physical characteristics of the fat supplement, chain length and the degree of saturation and the level of the supplemental fat in the diet. Also, the stage of lactation has shown to be a factor affecting the responses to FA supplementation in ruminants (Mashek et al., 2005; Bigner et al., 2009).

Given that lipids provided ME in the form of dietary fats or oils to the daily ration, the intake of forage DM, CP and that of NDF were lower during lipid infusions, as expected. No differences in apparent digestibility of feed components were found between treatments, with the exception of the anticipated 55% greater digestibility of ether extracts (EE) in lipid infusions vs. control infusion, and slightly higher (7%) digestibility of EE during CAM than during TAL infusions. The higher digestibility of EE after lipid infusions verify, in agreement with increased plasma NEFA concentration that the used infusion technique was successful in delivering the infusates to the abomasum.

The daily ME-balance across the treatment periods was slightly negative (-3.5, -4.2 and -4.6 MJ/d in CON, TAL and CAM infusions, respectively). The approximately 18% higher DMI during water vs. lipid infusions may have provided a greater availability of fuel precursors, mainly propionate, for hepatic gluconeogenesis (Holtenius et al., 2003; Janovick et al., 2011; Mann et al., 2016a; I). Consequently, the observed metabolic responses to IVGTT and IC of the Exp. 1 discussed in later chapters of this thesis may partially be affected by the reported difference in fuel supply between the experimental treatments.

CEI vs. HEI diet (II, IV)

Maintaining prepartal feed intake seems to be essential for a successful transition from late pregnancy to early lactation, as the voluntary DMI on MS-based diets has reportedly decreased up to 30% during the last 2 to 3 weeks of pregnancy (Hayirli et al., 2001, 2003; Grummer et al., 2004; Drackley et al., 2005). The experimental evidence of DMI decrease on GS–based diets is limited to only a few studies (Agenäs et al., 2003; Dewhurst et al., 2009; Little et al., 2016) showing not an equally extreme decrease in DMI as those reported on mainly MS-based and alfalfa forage diets. Consequently, in Exp. 2 (II, IV), a high-energy intake (HEI) GS-based diet supplying 144% of MER/d was fed during the 3-wk FODP, after which the daily energy intake was gradually restricted to the level of requirements by the day of expected calving. The intention was to investigate the combined effect of a high energy intake during the FODP and the subsequent decline in DMI occurring when cows approach calving as reported previously in abundantly fed overconditioned cows on MS-based diets (Grummer et al., 2004; Dann et al., 2006; Douglas et al., 2006). The DMI of controlled energy intake cows (CEI) was restricted to meet the daily MER. Concentrates were fed (30% of daily ME) throughout the CUDP period. The feed allowance was decreased linearly to simplify feeding in practice, and to ensure that the decline will occur in GS-based diets.

As designed, when HEI cows were kept on energy level of 144% of MER during the FODP, the difference in daily DMI and ME intake in between HEI vs. CEI during the FODP averaged 42% (Table 2), contributing to an average EB of 44.2 vs. 2.8 MJ/d in HEI vs. CEI, respectively (II, IV). In CUDP when HEI cows were forced to gradually decrease the DMI by limiting the daily allowance (II, IV), the magnitude of the decline in DMI of HEI was 30% between week 4 and parturition (IV), corresponding by volume to earlier findings on MS-based diets during the last 3 to 2 weeks of pregnancy (Grummer et al., 2004; Drackley et al., 2005). The observed difference in daily DMI and ME intake between HEI vs. CEI diminished to an average of 18.5% and 17% during the CUDP, respectively (IV) contributing to an average EB of 17.2 vs. 2.4 MJ/d in HEI vs. CEI.

CEI vs. HEI diet (III)

In loose-housing systems, group-fed dairy cows are prone to extra weight gain after drying-off. Dry cows easily overconsume energy up to 60% relative to requirements, with dietary energy content of 10 to 11 MJ/d in MS-based diets prepartum (Douglas et al., 2006; Dann et al., 2006; Janovick and Drackley, 2010). The DMI has remained more levelled as cows approach calving when fed diets with high-straw and low energy content (Dann et al., 2006) than when given high-energy diets during the CUDP (Grummer et al., 2004; Cardoso et al., 2013).

Experiment 3 (III) compared the effects of *ad libitum* allowance of GS (HEI) with a GS-based total-mixed ration (CEI) containing 40% wheat straw. The ME intake between HEI and CEI was around 39% greater in FODP (Table

2) and decreased to an average of 25% during the CUDP while total DM was 22% and 7.7% higher in HEI vs. CEI during the FODP and CUDP, respectively (III). These differences accounted to an average of 32% greater daily ME intake of HEI vs. CEI throughout the 8 wk dry period (144 MJ/d vs. 109 MJ/d) comprising to a total daily DMI difference of 16% (III). The observed very moderate dip in DMI in HEI cows (7% during the last 4 wk of pregnancy; III) does not support earlier work done mainly on MS-based diets, where high-energy intake during the dry period resulted in more dramatic dip in DMI prepartum (e.g., Dann et al., 2006; Janovick and Drackley, 2010; Ji et al., 2012, Mann et al., 2015).

Table 2. Effect of dry period energy allowance on dry matter intake and energy balance

	Experiment 2[1]			Experiment 3[2]		
Item[3]	**CEI**	**HEI**	**SEM[4]**	**CEI**	**HEI**	**SEM[4]**
PREPARTUM						
Weeks -6 to -4[1] (-8[2])						
Total DMI, kg/d	8.9	12.6[c]	0.26	11.8	14.4	0.73
ME intake, MJ/d	99	141[c]	3.3	105	146	7.28
ME-balance, MJ/d	2.8	44.2[c]	2.25	9.30	45.8	6.95
Weeks -3 to -1						
Total DMI, kg/d	9.7	11.5[c, f]	0.15	12.3	13.9	0.73
ME intake, MJ/d	109	128[c, f]	1.8	114	142	7.28
ME-balance, MJ/d	2.4	17.2[c, f]	2.22	7.3	29.8	6.95
POSTPARTUM						
Silage DMI, kg/d	10.1	10.7	0.56	11.2	11.3	0.54
Concentrate, DMI, kg/d	12.6[a]	12.1	0.16	10.9	11.3[a, d]	0.09
Total DMI kg/d	22.7	22.8	0.64	22.1	22.6	0.57
ME, MJ/d	254	253	6.2	262	270	6.56
ME-balance, MJ/d	-56.3	-47.6	7.9	-47.3	-56.4	8.69

[1] Exp. 2 (II, IV): CEI = 100% of ME requirements prepartum; HEI = 144% of ME requirements during weeks -6 to -4 followed by a gradual restriction of energy intake (119% of ME requirements) during the last 3 weeks of gestation.
[2] Exp. 3. (III): CEI = 108% of ME requirements prepartum; HEI = 141% of ME requirements prepartum, *ad libitum* allowance.
[3] Total DMI = Dry matter intake; ME = Metabolizable energy; ME-balance was calculated by subtracting the ME intake from estimated ME-requirements (Luke, 2017).
[4] SEM = Standard error of the mean.
[a] P < 0.10 diet; [b] P < 0.05 diet; [c] P < 0.01 diet; [d] P < 0.10 diet x time; [e] P < 0.05 diet x time; [f] P < 0.01 diet x time.

Effect of high vs. controlled energy intake prepartum (II-IV)

Limiting prepartal DMI by means of either restricting the daily amount of forage (II, IV) or by diluting the diet by addition of wheat straw for physical limitation to occur (III) was successful in controlling the DMI near to the level of requirements (Luke, 2019). The prepartal DMI in cows on controlled energy diets (III, IV) was well maintained even very near calving in accordance with earlier studies with similar energy intake levels on GS-based diets (Agenäs et al., 2003; Vickers et al., 2013). Current data (II-IV) is in alignment with earlier work demonstrating that controlling of energy intake either by limiting the

amount of TMR or GS (Agenäs et al., 2003; IV) or by modifying the composition of the diet (Rabelo et al., 2003; Dewhurst et al., 2009; Vickers et al., 2013; Little et al., 2016; III) are appropriate methods for preventing the dip in DMI near calving.

Indeed, it should be stressed that all cows in the CEI (III, IV) and those of HEI with decreasing energy allowance (IV) remained in positive EB throughout the dry period. Similarly, the moderate dip in DMI during the whole dry period of HEI cows in Exp. 3 (III) also point to a less important role of physical restriction on DMI decline during the CUDP, suggesting that other mechanisms adjust DMI particularly in the last weeks before parturition in (Agenäs et al., 2003; Dewhurst et al., 2010).

The current results (III-IV) point to a fact that the dip in DMI is not universal among cows on diets with varying forage species. More importantly, the dip in DMI in association with overfeeding of energy prepartum seem to be more moderate in GS-based diets (Agenäs et al., 2003; Dewhurst et al., 2009; Little et al., 2016; III) than in MS-based diets (Grummer et al.1995; Minor et al., 1998; Rabelo et al., 2003; Dann et al., 2006). In agreement with current results (III) whenever controlling of energy intake is implemented by adding straw or other bulky feeds into the MS-based rations (Janovick and Drackley, 2010; Litherland et al., 2012) or by restriction of energy allowance (Douglas et al., 2006; Janovick et al., 2011) the feed intake during the CUDP remains more constant.

The less pronounced dip in DMI before parturition in overfed cows on GS based diets than on MS-based diets is mostly due to the differences in nutrient composition between the forage types. In general, the NDF and protein content of MS are lower than that of GS, while starch content is higher in MS than in GS (Dewhurst et al., 2013). Consequently, intakes of TMRs based on MS are reportedly higher than on GS due to e.g. better NDF digestion and passage rate (Roche et al., 2006; Abrahamse et al., 2008; Dewhurst et al., 2013). Given that the nutrient composition of different types of forages modify rumen microbial ecology, VFA production, and rumination behaviour (Roche et al., 2006; Dewhurst 2013), the differences in any of these most likely explain the varied results of DMI between the current results (III, IV) and those of studies on MS-based diets.

Besides the physical restrain and inherent properties of forage types, a range of other factors affect the change in DMI during the CUDP (reviewed by Grummer et al., 2004; Roche et al., 2013; Drackley and Cardoso, 2014). These factors include both characteristics of animals (BCS, parity, breed, genetics) and dietary nutrient composition and, their interactions (Hayirli et al., 2002) as well as hormonal changes taking place during the CUDP. Of the endocrine adjustments, amongst other, increasing levels of estrogen (Jorritsma et al., 2003; Garnsworthy et al., 2008) and growth hormone (Bradford and Allen, 2008) are associated with decreasing EB, switching from body reserve accretion to mobilisation (Ingvartsen et al., 1999).

Effect of concentrate feeding period during the CUDP (II-IV)

As opposed to a non-existent decline in DMI of CEI (Agenäs et al., 2003; Mann et al., 2015; III, IV), Little et al., (2016) reported a reduction of 25% in DMI of cows fed only second cut GS when compared with cows given also concentrates during the 8 wk dry period, in agreement with earlier studies on GS diets (Keady et al., 2001; McNamara et al., 2003). All cows across the dietary treatments in the current experiments where given concentrates during the CUDP. In Exp. 3 (III) moderate amount of concentrates (1 to 2 kg/d) were given for a short period of time (last 12 ± 5 d (mean ± SD) of pregnancy), while in Exp. 2 (II, IV) all cows received 2 to 3 kg of concentrates (30% of the daily MER) during the CUDP. The decline in DMI for cows offered silage and concentrates in the study by Keady et al., (2001) and Little et al., (2016) was similar in magnitude (5%) to what was found in HEI (7 %) in the last 4 wk of pregnancy (III). The previous research investigating the decline in DMI on GS based diets supplemented with various concentrates during the DP report varied results, and less experiments have been done on GS than MS based diets. Compared with dry diets lower in non-fibre carbohydrates (NFC), diets with high starch content (i.e. over 40%) have caused a more dramatic decline in DMI shortly before calving (Grummer 1995; Minor et al., 1998; Rabelo et al., 2003; Van Saun 2014), whereas others have reported only modest effects of carbohydrate source on prepartal DMI (Smith et al., 2005).

4.1.2 FEED INTAKE AND ENERGY BALANCE POSTPARTUM

The effect of controlling energy intake before parturition to accelerate the increase of DMI in early lactation has been extensively studied lately (e.g. Dann et al., 2006; Douglas et al., 2006; Janovick and Drackley, 2010; Janovick et al., 2011; Roche et al., 2015). This is due to the fact that earlier studies on MS-based diets indicated that large decrease (up to 30–50%) in prepartal DMI and EB was associated with lower postpartal DMI and BCS loss (Grummer et al., 2004; Drackley et al., 2005), in disagreement with current findings (III, IV). Similarly, high energy diets (150 to 160% of energy requirement) fed during late pregnancy resulted in lower feed intake during the early lactation (Dann et al., 2006; Douglas et al., 2006; Janovick and Drackley, 2010). In contrast, restricting energy allowance by diluting MS-based diets with wheat straw during the dry period improved DMI and EB in very early lactation (Janovick and Drackley 2010; Janovick et al., 2011; Mann et al., 2015). A pooled analysis of 7 experiments showed a tendency of a positive effect of controlling the energy intake during the CUDP on DMI during the first month of lactation on MS-based diets (Cardoso et al., 2013). However, there is no consensus if similar effects on GS-diets exists (Ryan et al., 2003; Agenäs et al., 2003; Butler et al., 2011; Little et al., 2016).

Controlling energy intake by addition of chopped wheat straw to prepartum diet prevented effectively the excessive energy intake prepartum in multiparous cows (Janovick and Drackley, 2010; III). While the method for

addition of chopped wheat straw (III) to daily ration was successful in controlling the prepartal energy intake, no dietary effect on postpartal increment of DMI acceleration was found, as opposed to finding of Janovick and Drackley, 2010). This underpins the fact that differences in dietary composition, especially those between MS and GS, must be taken into consideration when comparing the results of studies conducted with animals on different forage sources.

The lack of effect on postpartal DMI (III, IV) is in agreement with other studies with moderate (30 to 40 %) overfeeding of energy during the dry period (Keady et al., 2001, 2005; Kokkonen et al., 2005; Butler et al., 2011; Mann et al., 2015; Vickers et al., 2013; Little et al., 2016). Although postpartum silage DMI and total DMI were unaffected by treatment (III, IV), concentrate DMI was higher in controlled fed cows (IV) and in *ad libitum* GS fed cows (III).

Controlling energy intake to meet or underscore the requirements before parturition indicated beneficial effects on the disease incidence, inflammatory status (Loor et al., 2013; Roche et al., 2017) and lactational performance mainly via improved DMI at early lactation. Also decreased hepatic lipid content (Dann et al., 2006) and increased liver oxidative capacity and decreased lipogenesis has been reported (Litherland et al., 2011). Interestingly, the excessive positive EB achieved by *ad libitum* fed MS-based diets before calving was associated with more negative EB during the very early lactation (the first 10 DIM), after which the effect was absent when assessed until lactation week 8 (Dann et al., 2006). On the other hand, cows on GS-based diets given surplus energy prepartum had a more pronounced and prolonged negative EB during the first 8 wk of lactation than cows whose energy intake was controlled prepartum (Agenäs et al., 2003).

Studies with addition of concentrates to CUDP diets have shown variable effects on postpartal DMI. Postpartal DMI increased when cows had *ad libitum* access of GS and 3 kg/d of concentrate during the CUDP with a 60% higher DMI compared with *ad libitum* access of 75:25 mixture of GS and straw (McNamara et al., 2003). Conversely, *ad libitum* fed medium quality GS with concentrates during the 8 wk dry period did not affect total DMI after parturition (Little et al., 2016).

4.1.3 PRODUCTION RESPONSE

When GS-based prepartal diets were compared with mixture of GS and straw diets, the milk yield was higher on pure GS diets (McNamara et al., 2003; Ryan et al., 2003) in agreement with the current results indicating greater milk yield in HEI than in CEI (III). However, in the earlier studies, the greatest dietary influence on milk production performance was observed during the early weeks of lactation, whereas in the current studies the differences in milk yield were evident only after the first 4 weeks of lactation (III, IV). The greater milk yield of CEI in Exp. 2 (IV) with controlled intake of moderately digestible GS throughout the 6 wk dry period (IV) was observed from wk 4 onwards, while

in Exp. 3 (III) the milk yield of HEI cows on *ad libitum* GS diet was 11.5% greater from week 5 onwards. The tendency for lower milk yield in HEI than in CEI (IV) is consistent with the study by Tesfa et al. (1999), showing that increasing the energy intake of dry cows with concentrate decreased milk yield in GS-based diets. As opposed to former, addition of concentrates on GS-based diets of dry cows improved milk production in the early lactation period (McNamara et al., 2003), while others reported no effect of dry period energy level on milk yield on GS-based diets (Keady et al., 2001; Agenäs et al., 2003; Kokkonen et al., 2005). Finally, in several studies on MS-based diets showing positive effects of controlled EI during the dry period on DMI, no effect on milk yield was reported (Dann et al., 2006; Douglas et al., 2006; Janovick and Drackley, 2010).

The varied responses are most likely attributable to differences in accretion and mobilisation of body reserves. For instance, in two of the previous studies the initial BCS at dry off was below 2.75 (McNamara et al., 2003; Ryan et al., 2003) as opposed to an initial BCS of 3.4 to 3.7 of animals in the current studies (III, IV). Most likely the higher milk yield observed in former studies was due to a greater increase in BCS from dry-off until calving in cows fed GS (McNamara et al., 2003; Ryan et al., 2003). One explanation for the lower milk yield in HEI (IV) may be that the induced 30 % decline in DMI with a moderate concentrate feeding (and no change in EB) during the CUDP was not optimal for the cow and caused metabolic inflexibility in the subsequent lactation while a more stable EB near calving in CEI (IV) was more advantageous to early lactation performance. In alignment with this, large changes in either DM or energy intake were associated to lower milk yield postpartum (Grummer et al., 2004; Drackley et al., 2005). On the other hand, allowing for *ad libitum* energy intake of second-cut GS with a scanty and short concentrate feeding period (III) seemed to have beneficial effect on subsequent milk production after the first month of lactation, when compared with CEI (III). The results are in agreement with studies on both MS- and GS-based diets (Agenäs et al., 2003; Janovick and Drackley, 2010).

The observed lower milk yield of CEI (III) may possibly be a carry-over effect of the prepartal diet composition. The common element of the two studies (Exp. 2 and 3; III, IV) conducted with genetically similar animals from the same herd was the slightly lower intake of concentrates during the early lactation in those animals producing less milk after the first month. In experiment 2 (IV) the concentrate feeding period lasted in average for 24 ± 5 d (mean ± SD) and was more intense (2 to 3 kg/d, 30 % of ME), while in experiment 3 (III) all cows received a very moderate amount of concentrates (1 to 2 kg/d) for a short period of time (12 ± 5 d; mean ± SD). The scarce concentrate feeding and dilution of prepartal diet with wheat straw to control the energy intake may have affected the rumen adaptation mechanism of CEI cows in experiment 3 (III), while gradual limiting of the daily amount of DMI of HEI cows (IV) may have induced alterations in the rumen milieu and nutrient supply.

Previously, it has been reported that as cattle transition from mainly forage-based diets to higher levels of concentrates, the rumen papillae increase both in size and in absorptional surface area, and undergo structural and functional changes (Odongo et al., 2006; Steele et al., 2011). The former may insinuate that the CEI diet with a short concentrate feeding (III) resulted in a too low starch or NFC intake to stimulate rumen papillae growth during the dry period in order to maximize the adaptation to a concentrate rich lactation ration after parturition. In contrast, higher contents of digestible carbohydrates, such as starch, enhanced the overall VFA absorption capacity via rumen epithelial papillae development (Goodlad, 1981; Dirksen et al., 1985; Aschenbach et al., 2010). However, the observed positive effects of inclusion of non-structural carbohydrates (NSC) to CU dry cows' diets on the development of rumen papillae were present when the inclusion of NSC was compared to low-quality forage diets (Dirksen et al., 1985). Indeed, inclusion of barley grain to dry cows' ration containing GS as a forage source showed no positive effect of development of the rumen papillae (Andersen et al., 1999) suggesting that other factors apart from the dietary components play a role in the modifications of rumen development.

4.2 TISSUE ACCRETION AND MOBILISATION

4.2.1 BODY COMPOSITION PREPARTUM

In Exp. 2 (II, IV), the moderate overfeeding of energy in the FODP together with gradual restriction of energy in CUDP did not affect BCS or BW (Table 3) in comparison to controlled energy intake with limited amount of GS (3.7 vs. 3.8 in HEI vs. CEI). In contrast, larger and significant increases in prepartal changes of BCS (0.4 vs. 0.1 units in HEI vs. CEI) and BW (1.4 vs. 0.8 kg/d in HEI vs. CEI) were associated with *ad libitum* feed allowance of GS in Exp. 3 (III) when compared with CEI intake of a mixture of GS and wheat straw.

The higher BW and BCS gain in Exp. 3 than in Exp. 2 might have been partly due to a longer duration of experimental dry period treatments in Exp. 3 (III). Further, the differences may be explained by differences in EB between the dietary treatments. HEI cows in Exp. 3 (III) remained in more positive EB than CEI cows (29.8 MJ/d vs. 7.34 MJ/d) throughout the CUDP (III), while a more moderate difference in EB between HEI vs. CEI diets in Exp. 2. (II, IV) was found in CUDP (17.2 MJ vs. 2.4 MJ/d, in HEI vs. CEI). The proportion of concentrates (II, IV) or the amount of concentrate (III) in the ration was equal across the cows within experiments during the CUDP, while the duration of the concentrate feeding period varied considerably (24 ±5 vs. 12 ± 5 d in IV vs. III), which may have contributed to the observed differences in tissue accretion. The small BW and BCS changes across the animals in both Exp. 2 and 3 (II-IV) were unexpected. A possible factor contributing to the relatively small increase in BW gain compared to the level of overfeeding in HEI may be

underestimation in calculation of maintenance requirement. The current energy recommendations for dry cows are most likely underestimated (Mandok et al., 2013; Kokkonen and Vanhatalo, 2014) and may thus explain the lower than expected BW gain during the dry period (II-IV). The lack of difference in BCS changes pre-calving (II, IV) may partly arise from insensitivity of the condition scoring system. The recommendation for an optimal BCS at calving for cows independent of production system is from 3.0 to 3.5 (5-point scale; Hayirli et al., 2002; Roche et al., 2009, 2015; Drackley and Cardoso 2014). This is due to the findings that cows with a higher BCS at parturition, are in greater risk for increased loss of BCS in early lactation increasing the risk for hepatic lipidosis, ketosis and disease incidence (Roche et al., 2015). In the current study the cows were already at this upper limit at dry off (II-IV). The results indicate that a dry period of 6 to 8 weeks was not sufficient to induce any dramatic changes in the BCS on GS-based diets in cows with optimal BCS at dry-off. Collectively, the results underpin that the adjustments for a targeted BCS at parturition should be put into practice in good time before the dry-off.

Table 3. Effect of dry period energy intake on body composition

	EXPERIMENT 2[1]			EXPERIMENT 3[2]		
ITEM[3]	**CEI**	**HEI**	**SEM[4]**	**CEI**	**HEI**	**SEM[4]**
PREPARTUM						
Initial BW, kg	694	692	20.8	740	725	39.0
BW at parturition, kg	727	738	24.3	781	800	39.3
BW change, kg/d	1.1	1.3	0.18	0.8	**1.4**[b]	0.16
Birth weight of the calf, kg	47.0[a]	40.7	2.25	41.2	41.0	1.73
Initial BCS, kg	3.7	3.6	0.10	3.5	3.4	0.20
BCS at parturition	3.8	3.7	0.14	3.7	3.8	0.20
BCS change	0.1	0.1	0.14	0.1	0.4[a, d]	0.08
POSTPARTUM						
Initial BW, kg	655	681	20.8	719	718	39.8
Final BW, kg	632	650	18.9	700	692	35.3
BW change, kg/d	-0.4	-0.6	0.17	-0.6	-0.8	0.26
Final BCS	2.7	2.8	0.17	2.9	3.1	0.21

[1] Exp. 2 (II, IV): CEI = 100% of ME requirements prepartum; HEI = 144% of ME requirements during weeks -6 to -4 followed by a gradual restriction of energy intake (119% of ME requirements) during the last 3 weeks of gestation.
[2] Exp. 3. (III): CEI = 108% of ME requirements prepartum; HEI = 141% of ME requirements prepartum, *ad libitum* allowance.
[3] BW = body weight; BCS = Body condition score [scale 1–5; Edmonson et al. (1989)]; PREPARTUM: Initial BW and BCS = measured on d -42 (II, IV) and on d -56 (III) relative to parturition; BW and BCS at parturition = measured -7 d (II, IV) and -5 d (III) relative to parturition; BW and BCS change = from -42 d to -7 d (II, IV) and from -56 d to -5 d (III) relative to parturition; POSTPARTUM: Initial BW and BCS = measured on d 1 after parturition; Final BW and BCS postpartum = measured on d 56 d after parturition; BW change = from 1 d to d 56 d after parturition.
[4] SEM = Standard error of the mean.
[a] P < 0.10 diet; [b] P < 0.05 diet; [c] P < 0.01 diet; [d] P < 0.10 diet x time; [e] P < 0.05 diet x time; [f] P < 0.01 diet x time.

4.2.2 BODY COMPOSITION POSTPARTUM

A recent meta-analysis showed that cows fed high-energy diets during the CUDP lost more BW during the first 6 wk postpartum than cows whose EI was controlled (Cardoso et al., 2013). Unlike studies showing decreased BW change and NEFA concentrations postpartum in dairy cows with energy restricted TMR during the dry period (Douglas et al., 2006; Janovick and Drackley, 2010; Janovick et al., 2011), no differences in BW and BCS losses (Table 3) or plasma NEFA concentrations were evident after parturition (II-IV). It seems that the beneficial effect of energy restriction during the dry period is not universal in all diet types (i.e. MS vs. GS), and may again be related to differences in nutrient composition of the diets (see p. 33).

The higher energy intake throughout the dry period (III, IV) may have accreted more visceral fat not detectable by BCS (Drackley et al., 2014). The increment of adiposity and the enlargement of adipocytes may affect the lipolytic signalling and insulin resistance in adipose tissue *in vivo* and *in vitro* (Jocken and Blaak, 2008; De Koster et al., 2016b). Moreover, it was shown that even a moderate overfeeding of energy during the dry period, without no visible signs of overconditioning, resulted in transcriptional modifications that predispose cows to fatty liver (Loor et al., 2006). Indeed, the results of lipidomic profiles of the same cows are in line with the former studies, as HEI cows in Exp. 2 (III) had greater concentrations of ceramides in the adipose tissue and displayed an opposite change of phospholipid profile after the parturition compared to CEI (Qin et al., 2018). The increase of sphingomyelins in the IV) potentially reflected the different magnitude of insulin resistance in the overfed cows compared to the cows on controlled-energy diet (Qin et al., 2017, 2018).

The body mobilisation in early lactation has been suggested to be related to an insufficient energy intake to support the mammary demands in in early lactation. This may, however, explain only partially the mechanism, as increasing the dietary energy content above the requirements in early lactation did not reduce mobilisation of body reserves (van Knegsel et al., 2005; Roche et al., 2009). The strong correlations (R^2 = 0.82 and 0.78 in Exp. 2. and 3, respectively) between BCS at dry-off and at 5 d prior to parturition across the treatments (III, IV) indicate that the role of the dry period energy level was minor in determining BCS at parturition. Indeed, dairy cows have an inherent level of body reserves toward which their metabolism is aiming during late lactation and in the dry period to restore the genetically predestined BCS (Friggens et al., 2004).

4.2.3 BIRTH WEIGHT OF THE CALVES

The prenatal growth of the calf may be affected by several factors including the diet composition and the dietary energy source. For instance, birth weight of calves was greater on a starch-based diet than on a more fibre-rich gestation diet (Loerch, 1996). In the current study, HEI cows tended to have in average

15% lighter calves (6.3 kg difference) than CEI cows in Exp. 2 (IV), which may have masked some of the difference in maternal weight gain. Lighter calves in HEI were unexpected, as earlier studies typically reported no effect of prepartal EI on calf weight with energy allowance ranging from 75 to 150% of MER in agreement with current results in Exp. 3 (Tesfa et al., 1999; Douglas et al., 2006; Janovick and Drackley, 2010; Little et al., 2016; III). The calf weights across the treatments in Exp. 3 (III) were in average on the same level as those of HEI in Exp. 2 (IV) and those of Little et al. (2016) in cows offered GS only or GS supplemented with concentrates during the whole dry period. In Exp. 3 (III) a less intense and a shorter concentrate feeding period was applied in CUDP, which may have contributed to the discrepancies between the studies (III, IV). Also, the differences in duration of the dry period feeding between the experiments and the decreasing energy allowance applied in Exp. 2 for HEI cows may explain the observed differences. Finally, the small number of animals used in current studies (III, IV) do not allow to draw any further conclusions on impact of energy level on calf weights.

4.3 PLASMA METABOLITE AND HORMONE CONCENTRATIONS

The alterations in transition period EB are reflected by changes in circulating insulin and glucose concentrations (Holtenius et al., 2003; Douglas et al., 2006; Janovick et al., 2011; Mann et al., 2016a; II-IV; Table 4). Simultaneously, the degree of adipose tissue mobilisation and hyperketonemia are increased, increasing circulating NEFA and BHB concentrations in early lactation (Dann et al., 2006; Janovick et al., 2011; Mann et al., 2016a; II-IV). These changes are observed as a response to adaptative mechanisms of reproductive and endocrine systems. The range of variation between individuals in the initiation of the adaptation mechanisms before parturition is reportedly large (Jorritsma et al., 2003; Kessel et al., 2008; van Der Drift et al., 2012; Weber et al., 2013). Overconditioning during the dry period may have detrimental effects on the metabolism and performance of the cows. In the current experiments, the effect of prepartal energy level on observed changes in plasma metabolite and hormone concentrations during the transition period were mostly observed prepartum and reflected the observed differences (or absence of differences II, IV) in EB and BCS changes (III), while dietary carry-over effects in early lactation were minor (II-IV). Overconditioning and the concomitant increase in prepartal insulin concentration (II-IV) may induce changes in adipose tissue sensitivity to insulin signalling (Ji et al., 2012; Selim et al., 2015). These changes may further accelerate adipose tissue mobilisation and increase ketogenesis shortly postpartum (Dann et al., 2006; Janovick et al., 2011; Mann et al., 2016a).

4.3.1 EFFECT OF INDUCTION OF HIGHER PLASMA NEFA

The attempts to mimic the negative EB of early lactating cows by induction of hyperlipidemia can be implemented by several means. In dairy cows feed restriction, either alone or in combination with abomasal or intravenous lipid infusions are typically used to investigate the relationship between negative EB and insulin resistance. Similarly, agents that inhibit lipolysis have been used in dry cows to compare the higher NEFA vs. lower of NEFA on insulin resistance (Pires et al., 2007a, 2007b, 2008; Schoenberg and Overton 2011; Schoenberg et al., 2012).

In the current Exp. 1 (I) a daily amount of 430 g of of beef tallow (TAL) or camelina oil (CAM) was infused into the abomasum to induce higher circulating NEFA levels. Infusions of lipids caused an expected elevation on plasma NEFA levels, as per experimental design, however, lipid source did not have an effect on plasma NEFA concentration (I; Pires et al., 2008). The achieved 50% higher NEFA concentrations in lipid treated cows (0.28 and 0.25 in CAM and TAL) than in CON are similar to those observed by controlling the energy intake (CEI) to the level of requirements or below in GS-based diets during the close-up dry period (CUDP); Holtenius et al., 2003; Kokkonen et al., 2003, 2004; II, III, IV). Similarly, the achieved NEFA levels of water infused cows (I) equal those observed in cows given surplus energy during the dry period with more positive EB (Holtenius et al., 2003; II, III, IV). The achieved NEFA levels show that the experimental induction of increased NEFA level in dry pregnant cows was an appropriate method for modelling the increment of NEFA levels. The infusions did not affect basal plasma glucose, insulin and BHB concentrations at d 5 of infusion (I).

The FA composition of TAL resembled that of the bovine adipose tissue (Smith et al., 1978) and comprised mainly of C16:0; C18:1, C18:2 (86% of total FA) in agreement with earlier studies (Mashek et al., 2005; Pires et al., 2007b; Brickner et al., 2009). Camelina oil had a high C18:3n-3 content (37% of total FA), with lower C18:3n-3 content than that of linseed oil used in earlier studies (50% of total FA; Pires et al., 2007b; Brickner et al., 2009). Infusion of lipids (CAM or TAL) altered plasma total long-chain fatty acid (LCFA) profiles on d 5 of experimental periods. All FA detected, with the exception of C18:2, C18:3, and C20:2 were significantly differently represented in plasma of cows following lipid infusions than after CON infusion. Compared to TAL, infusion of CAM increased the proportion of plasma PUFA by 20 percentage units, while C18:3n-3 content increased after CAM by 10.3 percentage units compared to TAL. Infusion of CAM decreased the percentages of C14:0, C16:0, C16:1, and C18:1 in plasma whereas infusion of TAL increased the proportion of SFA and monounsaturated FA of which the percentage of C16:0 and C18:1 increased by 5.7 and 13 percentage units when compared with CAM. Previously, in fed, nonlactating, nongestating cows infused with linseed oil or with TAL for 5 d, similar changes were reported (Pires et al., 2008).

Table 4. Effect of dry period energy intake on plasma metabolites and hormones

	EXPERIMENT 2[1]			EXPERIMENT 3[2]		
ITEM[3]	**CEI**	**HEI**	**SEM[4]**	**CEI**	**HEI**	**SEM[4]**
PREPARTUM						
Glucose, mmol/l	4.4	4.3	0.09	3.8	4.0[b,d]	0.06
Insulin, µIU/ml	15.9	24.2[c]	3.34	14.4	20.1[a,d]	
Glucagon, pg/ml	117.5	144.5		128.2	140.8	
Glucagon/insulin, mol/mol	0.29	0.26	0.027	0.37	0.36	
NEFA, mmol/l	0.24[b]	0.18	0.019	0.15[d]	0.12	
BHB, mmol/l	0.88[d]	0.78 d	0.054	0.61	0.67[c]	0.018
3-MH, µmol/l	6.82	6.36		7.48[a]	5.80	0.20
POSTPARTUM						
Glucose, mmol/l	3.4	3.3	0.13	3.2	3.4[a]	0.09
Insulin, µIU/ml	8.6	11.1	2.70	8.3	9.2	
Glucagon, pg/ml	153.0	142.1	12.94	144.9	131.6	
Glucagon/insulin, mol/mol	0.99	0.88	0.209	0.72	0.61	
NEFA, mmol/l	0.49	0.46	0.045	0.35	0.38	
BHB, mmol/l	1.47	1.50		1.32[e]	1.12	
3-MH, µmol/l	8.66[a]	7.89	0.558	9.28	8.78	0.819

[1] Exp. 2 (II, IV): CEI = 100% of ME requirements prepartum; HEI = 144% of ME requirements during weeks -6 to -4 followed by a gradual restriction of energy intake (119% of ME requirements) during the last 3 weeks of gestation.
[2] 3. (III): CEI = 108% of ME requirements prepartum; HEI = 141% of ME requirements prepartum, *ad libitum* allowance.
[3] NEFA = Nonesterified fatty acids; BHB = β-hydroxybutyric acid; 3-MH = 3-metylhistidine.
[4] SEM = Standard error of the mean.
[a] P < 0.10 diet; [b] P < 0.05 diet; [c] P < 0.01 diet; [d] P < 0.10 diet x time; [e] P < 0.05 diet x time; [f] P < 0.01 diet x time.
Statistical analyses conducted on log-transformed data.

4.3.2 PLASMA GLUCOSE AND INSULIN PREPARTUM

When dairy cows are fed either according to the requirements or below, most often glucose levels are not affected by dietary energy prepartum (Kunz et al., 1985; Rukkwamsuk et al., 1999a; Rabelo et al., 2005; IV). The absence of effect is because of the strict homeostatic control. As opposed to former, the concentration of plasma glucose was higher in HEI than in CEI before parturition, the difference being greatest during the last 2 wk of pregnancy (III). After parturition, the HEI cows tended to have higher glucose concentrations than CEI cows (III). In agreement, cows fed high energy diets during the entire dry period had higher prepartal concentration of basal glucose than cows fed controlled energy (Douglas et al., 2006; Schoenberg and Overton, 2011; Mann et al., 2016a; III). The higher blood glucose concentration in HEI (III) was potentially a result of increased availability of propionate for gluconeogenesis in the liver (Janovick et al., 2011; Mann et al., 2016a) as the CEI with high amounts of straw may have supplied less precursors for gluconeogenesis. The less positive EB of HEI, especially during the CUDP, probably accounted for the lack of such effect in Exp. 2. (IV) when the energy intake was gradually decreased.

Correspondent with higher glucose levels (III), *ad libitum* allowance of GS (III) tended to induce greater basal insulin secretion during the prepartal period when compared with CEI. Similarly, in Exp. 2. (IV) the average insulin concentration of HEI tended to be higher than in CEI in prepartal period. These findings complement with recent studies showing that energy overfeeding during the dry period resulted in higher prepartum insulin concentrations compared with cows fed restricted energy (Holtenius et al., 2003; Douglas et al., 2006; Janovick et al., 2011). Higher basal insulin level is also a compensatory mechanism of impaired insulin sensitivity in peripheral tissues (Perley et al., 1966; Polonsky et al., 1988; Kahn et al., 1993), a phenomenon that precedes impaired glucose tolerance and insulin resistance (Ahrén and Pacini, 2004; Bergman, 2007). Given that insulin is the most important hormone that inhibits hepatic gluconeogenesis mainly by suppressing the expression of genes for the gluconeogenic enzymes (Barthel and Schmoll, 2003), an increment of insulin resistance in the liver would increase glucose output and insulin levels. Hence, it may be argued that higher plasma insulin concentration associated with overconsumption of energy is an indicator of impaired glucose tolerance and/or insulin resistance on either hepatic or systemic level. However, because the hepatic glucose output was not investigated in the current range of studies, the former hypothesis remains to be confirmed in future studies. Moreover, in ruminants, insulin has a relatively low suppressive impact on hepatic gluconeogenesis from propionate (Hostletter-Allen et al., 1994; Donkin and Armentano, 1995; Smith et al., 2008), due to the high metabolic priority for hepatic glucose production (Aschenbach, 2010).

4.3.3 PLASMA GLUCOSE AND INSULIN POSTPARTUM

The observed decrease in basal glucose concentration after parturition (III, IV; Table 4) agrees with other studies (Holtenius et al., 2003; Kokkonen et al., 2005) and is a consequence of the greater nutritional needs of mammary gland, resulting in greater whole-body glucose and NEFA turnover after parturition (Reynolds, 2003). The mammary gland requires approximately 2 – 3 times more energy (Bell, 1995; Drackley, 2001) and 2.7, 2.0, and 4.5 times more glucose, amino acids, and fatty acids than that of the gravid uterus during late pregnancy (Bell, 1995). The adaptation to massive need for glucose is facilitated by reduced insulin binding in adipose tissue (Vernon and Flint 1983; McNamara, 1997) stimulating the mobilisation of adipose tissue releasing NEFA for use as an alternate energy source, also in the non-insulin sensitive mammary gland (Collier et al., 1984).

In early lactation, the rate of hepatic gluconeogenesis increases rapidly (Bell and Bauman, 1997). The low insulin levels facilitate adaptation to the increased glucose requirements by changes in insulin signalling pathways, resulting in increment in hepatic gluconeogenetic enzyme activity (Graber et al., 2010; Zachut et al., 2013). This does not necessarily involve development

of hepatic insulin resistance during the periparturient period in dairy cows (Zachut et al., 2013), although overfeeding energy prepartum downregulated the expression of key enzymes associated with hepatic gluconeogenesis in transition period in cows used in the current studies (II-IV; Selim et al., 2014, 2015).

The tendency for higher plasma glucose in HEI after parturition (III) is inconsistent with the absence of differences in plasma glucagon concentration or glucagon to insulin ratio suggesting no differences in hepatic gluconeogenesis from amino acids and lactate (Aschenbach et al., 2010). The higher postpartal plasma glucose (III) is in disagreement with the findings of Selim et al. (2015) suggesting an attenuated increase of hepatic gluconeogenic activity from propionate in HEI vs. CEI early postpartum. The finding was based on downregulation of hepatic PCK1 mRNA expression in HEI but not in CEI at d 1 and d 7 of lactation when compared with d -8 (Selim et al., 2015).

Consistent with the current results (III, IV) prepartal energy intake had no effect on insulin concentration postpartum (Holtenius et al., 2003; Douglas et al., 2006). As opposed to current findings (IV), Cardoso et al. (2013) found that cows fed high energy in FODP on MS-based diets had lower insulin concentrations during the first two weeks of lactation than cows fed controlled energy diets. The results again reinforce that inherent differences between MS and GS may result in different outcomes on plasma metabolites and hormones after parturition.

4.3.4 PLASMA NEFA AND BHB PREPARTUM

Current results of Exp. 2. (IV) support the earlier observations showing that when dietary energy is restricted during the dry period, the plasma concentrations of NEFA may be greater than in diets allowing for more liberate energy intake and a more positive EB (Douglas et al., 2006; Janovick et al., 2011; Schoenberg and Overton, 2011 Cardoso et al., 2013). As shown in Exp. 2. (IV), the plasma NEFA typically begin to rise during the late CUDP in dairy cows (Kunz et al., 1985; Douglas et al., 2006; Cardoso et al., 2013). The lower NEFA of HEI than CEI (IV) may suggests increased lipid deposition in adipose tissue, although the lack of dietary effect on BW and BCS changes in the transition period does not support this. The former may also suggest that higher energy intake throughout the dry period may have accreted more visceral fat not detectable by BCS (Drackely et al., 2014).

In Exp. 3 (III), the higher but still moderate plasma BHB in HEI than in CEI cows before parturition is most likely a consequence of a greater ruminal butyrate production due to higher intake of GS and not an indicator of metabolic imbalance (Roche et al., 2013). The level of prepartal energy intake affected the onset of tissue mobilisation near calving in Exp. 3. The tendency for a more pronounced rise of plasma NEFA and higher plasma 3-MH in CEI than in HEI cows in the weeks preceding calving indicates that CEI cows initiated the mobilisation process at an earlier stage precalving (III). No such

effect was observed in Exp. 2. where animals were in a less positive EB in the dry period (IV).

4.3.5 PLASMA NEFA AND BHB POSTPARTUM

Prepartal energy level did not have an impact on plasma NEFA concentrations after calving (Tesfa et al., 1999; Winkelman et al., 2008; III, IV),) in agreement with the lack of differences in BW and BCS change after parturition (III, IV). In earlier studies, larger BW and BCS gains during the dry period were associated with accelerated lipid mobilisation, indicated by higher plasma NEFA concentrations after calving (Holtenius et al., 2003; Kokkonen et al., 2005; Douglas et al., 2006; Ji et al., 2012) or during the FODP (Cardoso et al., 2013). The lack of differences in plasma NEFA (III, IV) concentrations after calving suggest that energy level before calving had no major effect on the use of NEFA and energy-yielding substrates to spare glucose after calving (Drackley et al., 2001). The NEFA model indices obtained from IVGTT data also agree with these results, as prepartal energy level did not affect the indices postpartum (II, III, see chapter 4.3.5). The hypothesis that greater energy intake prepartum would increase basal NEFA due to overconditioning during the CUDP inducing a vicious cycle of adipose tissue mobilisation in early lactation was not confirmed by the current studies (II-IV). Previously, greater circulating NEFA in cows with high energy intake diet during the FODP were associated with greater disease incidence and higher hepatic lipid infiltration after calving (Cardoso et al., 2013). The high energy intake during the dry period and concomitant greater liver fat content may decrease hepatic gluconeogenic capacity (Rukkwamsuk et al., 1999b; Murondoti et al., 2004). In support of this, in Exp. 2 (II, IV) the CEI cows had higher hepatic gene expression of pyruvate carboxylase (PC) than HEI cows (Selim et al., 2014), suggesting improvement of gluconeogenic capacity.

In line with current findings (III, IV) no effect of dry period energy level was found on average BHB concentration on GS-based diets after parturition in studies by Tesfa et al. (1999) and Holtenius et al. (2003). Other studies with more dramatic differences in BCS and BW changes (Kunz et al., 1985; Kokkonen et al., 2005; Douglas et al., 2006) reported increased blood BHB after calving in association with dry period overfeeding. However, a temporal effect of increased plasma BHB in association with controlled energy intake (CEI in III) was found at wk 6 and 8. A plausible reason for the higher postpartal BHB in CEI (III) near peak lactation may be related to a compensatory mechanism for insufficient supply of glucose precursors. The slightly lower DMI of concentrates in cows with lower milk yield after the 4th week of lactation (CEI in III) reinforces the suggestion that glucose precursors were perhaps a more limiting factor after parturition in CEI than in HEI. Indeed, moderate ketogenesis (i.e. < 1.2 mmol/L of basal serum BHB) in early lactation may serve as an alternate energy supplying pathway for glucose insufficiency (Duffield 2000; Dirksen et al., 2012, MacArt et al., 2013).

Considering that ketone bodies (BHB and acetoacetate) are products of hepatic FA oxidation and that FA are derived from hydrolysis and breakdown in the adipose tissue, BHB is engaged in a negative feedback mechanism suppressing high levels of circulating NEFA, and hence also BHB (Taggart et al., 2005). Given that BHB have been shown to inhibit basal adipose tissue lipolysis *in vitro* in a dose-dependent matter in cattle (Van der Drift et al., 2013), the higher BHB concentration may have possibly prevented ketoacidosis and exhaustion of fat depots in the CEI cows in Exp. 3 (Rojas-Morales et al., 2016). The absence of differences in plasma NEFA concentration between dietary treatments towards the end of the experimental period (III) further reinforce the hypothesis, that indeed a feedback mechanisms may have possibly prevented toxic effects of high BHB levels on tissues by shutting down further release of additional NEFA, as evidenced earlier in dairy cows (Rukkwamsuk et al., 1998). The absence of dietary effect on liver triacylglycerol concentration of the same animals in Exp. 2 and 3 (Selim et al., 2014, 2015) further complement the current results showing that dry period energy level did not have a major effect on hepatic fatty acid oxidation capacity.

4.3.6 BACK FAT THICKNESS AND PLASMA 3-MH

Both protein and adipose tissue mobilisation initiates already before calving, at around 2 to 1 wk prepartum (Doepel et al., 2002; Kokkonen et al., 2005). Protein mobilisation may start as early as 4 wk before parturition in cows fed limited amount of protein in the diet (Van der Drift et al., 2012). Nevertheless, the additional energy supply from muscle protein breakdown during negative EB is more limited both in duration and in volume than that from adipose tissue mobilisation (Tamminga et al., 1997; Komaragiri et al., 1998; Van Knegsel et al., 2007) as the adipose tissue mobilisation normally extends up to around 8 wk postpartum (Doepel et al., 2002; Van Knegsel et al., 2007; Van der Drift et al., 2012).

Consistent with the former, a reduction of 25% in skeletal muscle diameter was observed across the cows between -12 d and 28 d to parturition (III). In contrast, no differences were found between dietary treatments in muscle diameter (III), complementing the absence of difference between treatments in BCS and BW (III, IV) and reinforcing that prepartal diets induced no major differences in body mobilisation after parturition. However, the measurement method of muscle diameter may have some limitations, as it is based on a subjective ultrasonic method with visual inspection.

Profiles of plasma 3-methylhistidine (3-MH) are used as an indicator of muscle protein breakdown in cattle (Van der Drift et al., 2012). The higher prepartal plasma concentration of 3-MH in CEI than in HEI (III) point to initiation of increased amino acid mobilisation in response to controlled feeding already in CUDP (III) in agreement with earlier studies (Doepel et al., 2002; Kokkonen et al., 2005). The tendency for higher postpartal 3-MH in CEI

than in HEI (IV) suggest further that limiting the daily amount of feed prepartum may have increased muscle tissue mobilisation in early lactation to support the need for hepatic gluconeogenic precursors (IV). Overall, the observed alterations in plasma 3-MH concentrations (III, IV) and the muscle thickness changes (III) across the dietary treatments indicate that protein mobilisation started near parturition and continued at least until approximately wk 4 of lactation (III). These findings are consistent with earlier studies reporting decrement of muscle diameter and increase of 3-MH levels during the early lactation (Reid et al., 1980; Van der Drift et al., 2012). Also, the differences of 3-MH changes between HEI and CEI (III, IV) suggest that cows whose dietary energy intake is controlled during the dry period may use proportionally more mobilized amino acids for hepatic gluconeogenesis in early lactation as suggested by others (Overton, 1999; Drackley et al., 2001). The earlier mentioned greater hepatic gene expression observed in CEI cows (Selim et al., 2014) in Exp. 2 (IV) support the former and suggest a promoted entry of endogenous substrates for gluconeogenesis as a response of controlled energy intake in the dry period (Aschenbach et al., 2010).

Lactate derived from increased skeletal muscle glycolysis and from activation of Cori cycle, is the main substrate for compensation of lack of propionate for hepatic gluconeogenesis in early lactation (Kuhla et al., 2011; Larsen and Kristensen, 2013). Among amino acids only alanine has a major role in contributing to liver glucose release, as suggested by Larsen and Kristensen (2013), while other muscle-derived amino acids are mainly used by peripheral tissues. Particularly in cows whose energy restriction was conducted by diluting the GS with wheat straw prepartum (III) it may be suggested that there was lack of gluconeogenetic precursors.

4.4 RESPONSES TO IVGTT AND IC

4.4.1 INSULIN SENSITIVITY AS ASSESSED BY IVGTT

The evaluation of the responses of plasma hormones and metabolites to metabolic challenges are demanding, as the dynamics of insulin and glucose are reciprocally regulated (Hayirli et al., 2001; Kahn et al., 2006). Similarly, plasma NEFA and glucose are metabolically competitive via a number of proposed mechanisms (Randle et al., 1963; Bergman et al., 1993; Bergman and Iyer, 2017). A range of studies with different assessment methods adopted mainly from human medicine are used to assess insulin resistance of glucose metabolism and insulin responsiveness of tissues during pregnancy and lactation in ruminants (reviewed by Opsomer et al., 1999; Schoenberg and Overton, 2010; De Koster and Opsomer 2013).

The IVGTT is based on a bolus infusion of glucose and frequent blood sampling during 2 to 3 hours to assess hyperglycemia, hyperinsulinemia and changes in blood NEFA concentrations. Magnitude of changes in blood

concentrations of glucose, insulin and NEFA give information on pancreatic insulin release, glucose disposal and insulin-dependent suppression of blood NEFA concentrations by inhibition of adipose tissue lipolysis (Hayirli, 2006; Boston and Moate, 2008; Grünberg et al., 2011; Schoenberg and Overton 2011). Given that the non-insulin-modified IVGTT used in the series of experiment discussed in this thesis (I-III) use a single dose of glucose, the observed changes in calculated parameters during IVGTT are discussed accordingly.

Following glucose infusion, decreased clearance rate, increased time to reach half-maximal concentration, and time to reach basal concentration, as well as incremental AUC reflect increased glucose intolerance and decreased insulin sensitivity (Kahn, 1978; Sano et al., 1991; Hayirli et al., 2001). Differences in peak glucose concentrations during an IVGTT are discussed as being implicative of changes in tissue insulin responsiveness (Kahn, 1978; Schoenberg et al., 2012), providing that insulin concentrations are similar between evaluated treatment groups during the IVGTT (Hayirli et al., 2001; Hayirli, 2006).

The IVGTT has been used extensively during the last 15 years in cattle to measure the relative changes in insulin resistance in calves and dry cows as well as in transition and lactating dairy cows (e.g. Kräft, 2004; Smith 2005; Pires et al., 2007b, 2008; Bossaert et al., 2008; Schoenberg et al., 2012; Weber et al., 2013; Marett et al., 2015; De Koster et al., 2016; Mann et al., 2016a, Weber et al., 2016). As this test fails to discriminate between insulin-dependent and insulin-independent glucose disposal during the test, some investigators have argued against the use of the test in early lactating dairy cows (Mann et al., 2016a; De Koster et al., 2016a, 2017). The arguments are based on the fact that the large insulin-independent glucose uptake by the mammary gland (Bauman and Currie, 1980; Debras et al., 1989; Bell and Bauman, 1997) may mask any dietary effects of the insulin-dependent changes during the test. However, unlike the HEC test, IVGTT together with MM can take into account the endogenous insulin secretion after the glucose bolus. Indeed, a major pitfall in interpretation of results derived from HEC test include the simultaneous infusion of both insulin and large amounts of exogenous glucose which are confounding factors, and partially encumber the determination whether the results are an outcome of insulin, glucose or both.

During a standard IVGTT the observed glucose concentration is a sum of glucose consumption by peripheral tissues, endogenous glucose production (representing mainly the hepatic output of glucose), renal glucose excretion, and intestinal glucose absorption (Pires et al., 2008). The assumption in the current experiments (Exp. 1-3) concerning insulin´s effect on hepatic output during the IVGTT (I, II, III) was that plasma insulin concentrations were high enough to inhibit endogenous glucose production during the first hour of IVGTT. In ruminants, maximal inhibition of hepatic gluconeogenesis is achieved at insulin concentrations ranging from 100 to 120 mU/mL (Brockman and Laarveld, 1986; Petterson et al., 1993). Because the peak

insulin concentrations across the treatments (I) and diets (II-III) were higher than aforementioned values, it was assumed that the glucose production in the liver was inhibited during the first hour of IVGTT (I, II, III). Thus, the observed clearance rate (CR) and AUC during the first 60 min of the challenge (AUC_{60}) were thought to represent both insulin-independent and insulin-dependent glucose uptake.

A confounding factor for IVGTT derived values of glucose net disposal is the fact that renal infiltration most likely occurs during the first hour of IVGTT. The range of renal threshold for glucose reported in adult cattle is from 5.4 to 11.2 mmol/l (Hostettler-Allen et al., 1994; Bernhard et al., 2012), and these values were exceeded during the first 30 min IVGTT (I-III), suggesting that estimated glucose disposal may have been masked by renal spill over of glucose. However, the proportion of glucose excreted in urine was probably relatively small because only 4 to 7% urine excretion was observed lately in dairy cows with higher infusion volumes (Grünberg et al., 2011)

The role of liver glucose production and the role of hepatic insulin resistance on the observed changes in glucose dynamics during the IVGTT may also affect the interpretation of the results, and hepatic residual output cannot be totally excluded (Hostettler-Allen et al., 1994). Similarly, renal glucose production has a small impact on the total glucose output, especially during the early lactation (Bell and Bauman, 1997). Considering that neither hepatic nor renal glucose output was measured in the current studies, little can ultimately be said about the role of hepatic insulin resistance. As insulin regulates hepatic insulin resistance by affecting several enzyme activities in transition period (Zachut et al., 2013; Selim et al., 2014, 2015) it is assumed that in hepatic insulin resistance stages the gluconeogenic capacity may be compromised (Selim et al., 2014), affecting CR and AUC of glucose during an IVGTT.

4.4.2 GLUCOSE DYNAMICS DURING THE IVGTT PREPARTUM

Excess energy intake during the dry period is associated with decreased glucose tolerance in dairy cows. Overconditioned cows were more insulin resistant than leaner cows in regard to glucose metabolism and glucose transport into adipose tissue in late pregnancy (De Koster et al., 2015; Jaakson et al., 2018).

In humans, it has been suggested that the release of NEFA from adipose tissue may be the single most critical factor in modulating insulin sensitivity (Kahn et al., 2006). The typically observed greater NEFA levels in obese and type 2 diabetic subjects are associated with insulin resistance of glucose and fatty acid metabolism accompanying both of these metabolic conditions (Boden, 1997; Reaven et al., 1998). Peripheral insulin resistance develops within hours of an acute increase in plasma NEFA levels in humans (Roden et al., 1996; Carpentier et al., 1999) and in only few days abomasal infusions of lipids in dairy cows (Pires et al., 2007a, 2008; I). Decreased glucose disposal

was observed in dairy cows after a 4-d fast (Oikawa and Oetzel, 2006; Schoenberg et al., 2012) to induce higher plasma NEFA concentrations. In comparison, improvement of both insulin-mediated glucose uptake and glucose tolerance was observed after treatment with antilipolytic agents reducing NEFA levels in humans (Santomauro et al., 1999) and in dairy cows (Pires et al., 2007b). An aggravated lipolysis may result in the accumulation of lipids in the liver and likely contributes to the increased susceptibility of metabolic disorders in periparturient dairy cows (Grummer et al., 2004; Drackley et al., 2005).

Effect of induced higher plasma NEFA (I)

In Exp. 1 (I) , a moderate increment of average basal NEFA measured at treatment d 5 (I) after 98 h of lipid infusion (0.17 vs. 0.27 mmol/L, in CON vs. lipid infusions, respectively) to a typically reported level of prepartal dry cows on GS-based diets (Holtenius et al., 2003; Kokkonen et al., 2004, 2005; II, III) decreased glucose disposal (CR of glucose) during IVGTT and IC (I) as shown in Table 5. The findings complement the reported impairment of glucose clearance in association with induced higher plasma NEFA in non-pregnant and pregnant dairy cows (Oikawa and Oetzel, 2006; Schoenberg et al., 2012). The impairment of glucose disposal after induction of higher NEFA was further confirmed by similar observed response during IC as evidenced by both higher nadir and decreased clearance of glucose in lipid infused than CON cows (I).

In Exp. 3. (III), the positive associations between basal NEFA and glucose AUC prepartum and between NEFA clearance and glucose AUC postpartum (III) refer to a reciprocal regulation between these two metabolites as well. In humans, fatty acids limit insulin-stimulated glucose utilization by several mechanisms (Randle et al., 1963; Randle, 1998; Bergman and Iyer, 2014). The Randle´s glucose-fatty acid cycle proposes that glucose and fatty acid compete for their oxidation in insulin-dependent peripheral tissues. Hormones that control adipose tissue lipolysis affect circulating concentrations of NEFA, which in turn control fuel selection in skeletal muscle by alteration in enzyme activities (Randle, 1998; Frayn et al., 2006; Sugden, 2007). The associations suggest an inhibitive role of higher NEFA on glucose disposal in stimulated conditions, as was verified by current data (I) in agreement with non-pregnant and pregnant dairy cows (Pires et al., 2007b, Schoenberg et al., 2012). In Exp. 1 (I), differences between fat sources were found on CR of glucose during IVGTT. Compared to TAL infusion, lower concentration of insulin (AUC of insulin) was needed to achieve similar glucose disposal than after CAM infusions in agreement with PUFA containing FA infusions with insulin sensitizing effect when compared with TAL (Pires et al., 2007b, 2008).

Table 5. Effect of abomasal infusion of lipids and prepartal energy level on insulin resistance as assessed by IVGTT prepartum

	EXPERIMENT 1[1]					EXPERIMENT 2[2]				EXPERIMENT 3[3]			
ITEM[4]	CON	TAL	CAM	SEM[5]	P-value[§]	CEI	HEI	SEM	P-value[†]	CEI	HEI	SEM[5]	P-value[‡]
Glucose													
Basal[1](mmol/l)	3.9	4.1	4.1	0.10	0.07	4.4	4.4	0.15	0.76 [c]	3.9	4.0	0.12	0.14
Peak (mmol/l)	18.6	17.6	17.4	0.44	0.02	17.0	17.1	0.60	0.87 [c]	19.4	19.2	0.37	0.71
AUC_{60}	418	401	417	12.6	0.57	-	-	-	-	404	385	20.0	0.22
AUC_{total}	617	609	664	43.7	0.69	441	440	16.1	0.94[c]	525	413	47.9	0.02[a]
CR_{60} (%/min)	1.74	1.48	1.34	0.133	0.03	1.7	1.7	0.11	0.72 [c]	1.3	1.4	0.12	0.83
T½[5] (min)	42.0	47.3	53.5	3.88	0.06	-	-	-	-	-	-	-	-
Insulin													
Basal (µIU/ml)	20.7	15.0	16.6	1.94	0.06	41.0	44.2	3.65	0.61 [c]	13.8	15.7	2.03	0.52
Peak (µIU/ml)	128	113	104	13.9	0.14	331	384	50.5	0.83 [c]	224	398	73.3	0.02[c]
AUC_{60}	4 592	4 064	3 165	572.0	0.02	9922	12743	1813	0.40 [c]	7920	13916	2622	0.03[b]
AUC_{total}	6 830	7 287	5 992	744.0	0.75	11741	15279	2435	0.99 [c]	10064	17762	3520	0.05[b]
CR_{60} (%/min)	0.10	0.40	0.50	0.17	0.04	0.8	0.6	0.21	0.36 [c]	-0.62	-0.72	0.11	0.40[b]
NEFA													
Basal (mmol/l)	0.11	0.21	0.23	0.025	<0.001	0.24	0.16	0.04	0.89 [a]	0.29	0.24	0.05	0.12[b]
Nadir (mmol/l)	-	-	-	-	-	-	-	-	-	0.10	0.08	0.01	0.32[a]
Decrement (mmol/l)	-	-	-	-	-	0.14	0.09	0.02	0.95 [c]	0.19	0.16	0.04	0.19[a]
AUC_{60}	-1.7	-1.0	0.8	1.6	0.41	-3.0	-2.3	0.97	0.11 [c]	-2.6	-2.3	1.1	0.83
AUC_{total}	-8.5	-11.9	-10.3	2.85	0.48	-15.7	-10.9	4.45	0.83 [c]	-20.0	-13.4	4.33	0.18
CR_{60} (%/min)	1.42	1.51	1.39	0.199	0.87	1.5	1.4	0.97	0.11	1.71	1.25	0.48	>0.10*
T½ (min)	51.2	47.1	71.4	14.9	0.64	-	-	-	-	-	-	-	-
NEFA model													
FFA0 (µmol/l)	-	-	-	-	-	243	161	37.3	0.45[c]	390	254	63.5	0.09[a]
SFFA (µmol/l min^{-1})	61	105	102	0.147	0.19	16.4	12.7	5.04	0.42 [c]	19.9	21.5	3.59	0.74
KFFA (min^{-1})	0.42	0.19	0.24	0.12	0.11	0.03	0.05	0.005	0.21[a]	0.03	0.04	0.006	0.09[a]
Latency, min	-	-	-	-	-	-	-	-	-	11.9	16.6	1.26	0.04

[1] Exp.1 (I): CON = abomasal infusion of water (98 h); TAL = abomasal infusion of tallow (98 h); CAM = abomasal infusion of camelina oil (98 h); [5] SEM = Standard error of the mean

[2] Exp. 2 (II): CEI = 100% of ME requirements prepartum; HEI = 144% of ME requirements during weeks -6 to -4 followed by a gradual restriction of energy intake (119% of ME requirements) during the last 3 weeks of gestation.

[3] Exp. 3. (III): CEI = 108% of ME requirements prepartum; HEI = 141% of ME requirements prepartum, *ad libitum* allowance.

[4] Basal = Average concentration at 15 and 5 min before IVGTT; AUC_{60} = Area under the curve during first 60 min of IVGTT; AUC_{total} = Area under the curve during 180 min (I-II) or 240 min (III) of IVGTT; CR_{60} = Clearance rate during first 60 min of IVGTT; $T_{1/2}$ = Time to reach 1/2 concentration; FFA0 = basal NEFA estimated by NEFA model analysis; SFFA = rate of entry of NEFA to the plasma pool; KFFA = fractional disposition rate of NEFA from the plasma pool; latency = time until NEFA concentration begins to decline.

[§] P-value for treatment effect (lipids vs. control); [†] P-value for treatment effect across transition period; [‡] P-value for treatment effect (CEI vs. HEI); [a] $P < 0.10$ day to parturition; [b] $P < 0.05$ day to parturition; [c] $P < 0.01$ day to parturition.

Effect of high vs. controlled energy intake prepartum (II-IV)
As opposed to publication I, the prepartal energy intake did not affect glucose dynamics in Exp. 2 (II), despite an equal difference in weekly plasma NEFA concentrations between HEI and CEI diets (0.18 vs. 0.24 mmol/l, respectively) to what was observed in publication I between CON and lipid infused cows. The varied glucose responses between the experiments (I vs. II) may reflect differences in physiological state of the cows during the IVGTT (i.e. the stage of the dry period). The absence of effect of prepartal dietary energy level on glucose and insulin dynamics was also reported in late pregnant and in transition dairy cows (Schoenberg and Overton, 2011; Schoenberg et al., 2012; Mann et al., 2016a). In contrast, the *ad libitum* energy intake of HEI in Exp. 3. (III), induced a compensatory insulin secretion in prepartal IVGTT, which led to a decrement in total glucose AUC preserving the peripheral glucose tolerance (III). As opposed to publications I and II, no significant difference in basal NEFA concentration was observed between HEI and CEI.

Altogether the results suggest that different mechanisms mediate glucose responses during an IVGTT after lipid infusions and after high energy intake. Most importantly, the 98-h lipid infusion did not induce changes in BCS and BW (I) and the animals were all in a slightly negative EB (I) while in publications II and III all cows remained in positive EB. The absence of BCS and BW change was evident in experiment 2. (II), while in *ad libitum* GS diet (HEI, III), the significantly greater increase in BW (75 kg vs. 40.8 kg) during the entire dry period most likely affected the observed responses in insulin and glucose dynamics (III). Secondly, it was lately suggested that overfeeding may induce only minor visible changes in BCS, while overfed cows most likely share common metabolic features with the obese human, that are beyond visual inspection (Drackley et al., 2014). The accretion of visceral fat depots, which drain directly to the liver (Kahn et al., 2006), may expose the liver for excess NEFA inducing hepatic insulin resistance. Given the greater activity to lipolysis and lower sensitivity to insulin in visceral than subcutaneous adipose tissue (Montague et al., 2000), visceral adipose tissue may further enhance insulin resistance and NEFA delivery to peripheral tissues. Indeed, studies showing deteriorated insulin sensitivity and responsiveness of glucose metabolism during metabolic challenges prepartum show that the decreased response was negatively associated with accumulation of adipose tissue as assessed by greater BCS (Holtenius et al., 2003; De Koster et al., 2015, 2016; Bogaert et al., 2018; Jaakson et al., 2018).

4.4.3 INSULIN DYNAMICS DURING THE IVGTT PREPARTUM

Insulin is the most important hormone inhibiting hepatic gluconeogenesis mainly by suppressing the expression of genes for the gluconeogenic enzymes (Barthel and Schmoll, 2003). In early lactation along with the abrupt increase in milk production, the rate of hepatic gluconeogenesis is rapidly enhanced (Bell and Bauman, 1997). The decreased insulin concentration (Figure 1)

shortly after parturition (Reist et al., 2003; Hammon et al., 2009; Weber et al., 2013; II, III) is the key factor in homeorhetic regulation of early lactation nutrient partitioning (Bauman and Currie, 1980). The high dietary energy level induced numerically higher prepartal insulin peak concentrations and CR of insulin when compared with controlled intake (Holtenius et al., 2003). In contrast, absence of differences in insulin response have been found more recently between high energy and controlled energy intake in cows in transition period (Schoenberg and Overton, 2011; Schoenberg et al., 2012; Mann et al., 2016a). In the former study the lack of effect on insulin response was evident despite greater increase in BW and BCS in high and controlled energy allowance. Similarly, only a weak association between BCS and insulin concentration was reported in lactating dairy cows (Bradford and Allen, 2007). Moreover, Schoenberg et al. (2012) showed dramatical decreases both in basal and glucose-induced insulin secretion in late pregnant dry dairy cows in response to feed-deprivation, suggesting that different mechanisms are mediating insulin response after feed restriction than in fed state. No consensus on the effects of overfeeding on insulin signalling during the transition period in dairy cows exists (Ji et al., 2012; Selim et al., 2015; Mann et al., 2016b), reflecting that several factors mediate the observed alterations in insulin responses.

In humans, transiently elevated NEFA have a tendency to enhance insulin secretion, whereas prolonged exposure to high NEFA tends to reduce insulin secretion (Rosen and Spiegelman, 2006). Similarly, a high-fat diet fed for 12 wk in dogs to model human Type 2 diabetes, induced insulin resistance within a week and decreased SI by 30% (Mittelman et al., 2000). The reduced SI was initially compensated by compensatory insulin secretion, and subsequently by decreased hepatic insulin clearance (Mittelman et al., 2000; Kim et al., 2007) in agreement with suggested mode of action in equine metabolic syndrome (Frank and Tadros, 2014).

Effect of induced higher plasma NEFA (I)

In Exp. 1 (I) compared with CON, the lower insulin response during the IVGTT after induction of higher NEFA resulted both from lower insulin secretion and from an increase in insulin clearance. In line with the current findings, a negative correlation was found between NEFA concentration and insulin AUC and peak concentration in transition cows, suggesting that elevated NEFA concentrations may interfere with glucose-induced insulin secretion (Bossaert et al., 2008). The results also suggest that even a short-term lipid treatment to induce higher plasma NEFA deteriorates insulin sensitivity of glucose metabolism in dairy cows, mainly by decreased insulin secretion. However, the increased insulin clearance after lipid treatments suggest that alterations in hepatic insulin extraction contributed to observed changes in insulin dynamics as well (I). Indeed, infusions of insulin sensitizing agents that decrease plasma NEFA in non-lactating, non-pregnant and pregnant dairy cows enhanced insulin sensitivity, as evidenced by improved

clearance of glucose with equal insulin responses during an IVGTT and IC (Pires et al., 2007a; Schoenberg et al., 2012).

In agreement with current results (I), Pires et al. (2008) postulated that plasma NEFA is at least partially a causal factor in insulin resistance in dairy cows in transition period, when plasma NEFA are normally elevated. The estimated values of insulin sensitivity from the MM (SI and DI) reinforce the findings and suggest that CAM rather than TAL had an insulin-sensitizing effect during IVGTT (I). Similarly, the lower insulin concentration during IC in CAM than in TAL led to the same clearance of glucose in both groups, gives further support to the insulin-sensitizing effect of CAM. In accordance with earlier work on ruminants (Pires et al., 2007b, 2008; Gingras et al., 2007, 2013), linseed oil and fish-oil, both rich sources of n-3 fatty acids, decreased insulin secretion and improved peripheral insulin sensitivity in dairy cows and steers (Gingras et al., 2007; Pires et al., 2008).

The reason for the insulin sensitizing effect of oils rich in C18:3n-3, is not clear but several theories have been proposed. Most likely the altered plasma profiles of major fatty acid groups mediated the observed responses in glucose and insulin dynamics after lipid infusions (I). The infused tallow comprised mainly of C16:0, C16:1 and C:18:1, increasing the total concentrations of these three atty acids in the plasma by 60% when compared with CAM (I). Given the greater insulin response to glucose after TAL than after CAM infusion, it is likely that the response is mediated by differences in the FA composition of the lipid sources (I) especially those of C16:0, C18:1. It is known that both palmitic acid (C16:0) and oleic acid (C18:1) alter insulin secretion by causing β-cell dysfunction (Maedler et al., 2003; Chavez and Sumners, 2012). The greater proportion of monounsaturated FAs may have also induced an inflammatory process promoting the synthesis of ceramides, as reported in humans (Chavez and Summers, 2012), and dairy cows (reviewed by Mc Fadden and Rico, 2019). Worthy of note is that in companion paper to Exp. 2 (II, IV), hepatic sphingomyelin lipid classes were greater in HEI than in CEI with concomitant increment of ceramides in adipose tissue before parturition (Qin et al., 2017). Ceramide accumulation in the muscle, especially in the presence of SFA, is known to induce insulin resistance via interruptions in insulin signalling pathway (Summers and Goodpaster, 2016) suggesting that these lipid classes may mediate responses in glucose metabolism. Similarly, the ceramides may be hypothesized to be partial mediators of the impairment of insulin signalling after lipid infusions (I).

Effect of high vs. controlled energy intake prepartum (II-IV)

The basal insulin level immediately before the prepartal IVGTT across the diets in Exp. 2 (II) was 2.7-fold greater than those in Exp. 1 (I) and Exp. 3 (III) (43 vs. 17 and 15 mIU/mL in II vs. I and III, respectively) (Table 5). The insulin concentrations in I and III are in agreement with earlier reported values in GS-based diets (Holtenius et al., 2003). Given that diets were all GS-based and that cows were in quite similar physiological state, especially in Exp. 2 and 3.

(II, III) in CUDP, the difference was unexpected. The most obvious reason for the greater insulin is attributable to a shorter fasting period (1 h vs. 3 h in II vs. I and II, respectively) at the time of the challenges and to the fact that in Exp. 2 (II) cows were fed concentrates by the time of the prepartal challenge, while in I and III only forages were fed. Most likely the hepatic uptake of propionate for glucose production before the challenges were increased due to concentrate feeding, reflected by greater basal glucose concentrations immediately before the IVGTT (4.4 vs. 4.1, and 4.0 mmol/L across all cows in II vs. I and III, respectively).

The HEI diet in Exp. 2. (II) did not affect glucose metabolism and insulin response (Figure 1; A) during the IVGTT when compared with CEI analogous with earlier findings on MS-based diets (Schoenberg and Overton, 2011; Schoenberg et al., 2012; Mann et al., 2016a). The lack of effect on glucose and insulin dynamics (II) correspond with the absence of differences in prepartal BCS and BW change between dietary treatments (II). In Exp. 3 (III) the greater insulin response on HEI, as evidenced by an approximately 80% greater peak and AUC of insulin than on CEI diets (Figure 1; B) suggest an altered insulin sensitivity of peripheral tissues in response to overfeeding of energy (Kahn, 1978; Kahn et al., 1993). The subsequently smaller AUC of glucose, however, indicates that compensatory insulin secretion adapted to alterations in insulin sensitivity of the peripheral tissues, preserving glucose tolerance. In this study the higher prepartal energy intake induced greater body accretion before parturition (III).

In humans and other animals, any environmental change (e.g. in response to overconditioning) in insulin sensitivity is known to be compensated by an increase in insulin secretion in response to glucose (Bergman, 1989; Kahn et al., 1993; Mittelman et al., 2000; Kim et al., 2007; Frank and Tadros, 2014). In agreement with the former and with current results (III) a more pronounced insulin response to IVGTT at −21 d in cows with BCS >3.75 (1–5 scale) was found compared with leaner cows (BCS <3.0) on GS- and hay-based TMR (Jaakson et al., 2018). The current results (II, III) are in line with earlier studies showing that insulin response is closely associated with overconditioning in dairy cows (Holtenius et al., 2003; De Koster et al., 2015; Jaakson et al., 2018). As opposed to current results (III), the overconditioned cows had larger glucose AUC than the leaner cows, indicating a higher degree of insulin resistance without an appropriate pancreatic compensatory capacity (Bogaert et al., 2018; Jaakson et al., 2018).

The discrepancies between studies may arise from differences in feed composition and breed and production level, and from dissimilarities in initial and achieved BCS between treatments, and in the amount of glucose infused. Most importantly, the timing of the challenge relative to parturition is a factor contributing to differences in reported results. In Exp. 1 (I) the cows were in FODP while in Exp. 2. (II) and 3. (III) the prepartal IVGTT was conducted in CUDP, during which homeorhetic adaptations most likely modulate the insulin response of the glucose metabolism (Bell and Bauman, 1997). Indeed,

the significant effect of the interval between the prepartal IVGTT and the day of calving for peak concentration and AUC of insulin reinforce the former indicating the decrement of insulin response towards the due date across the animals (III).

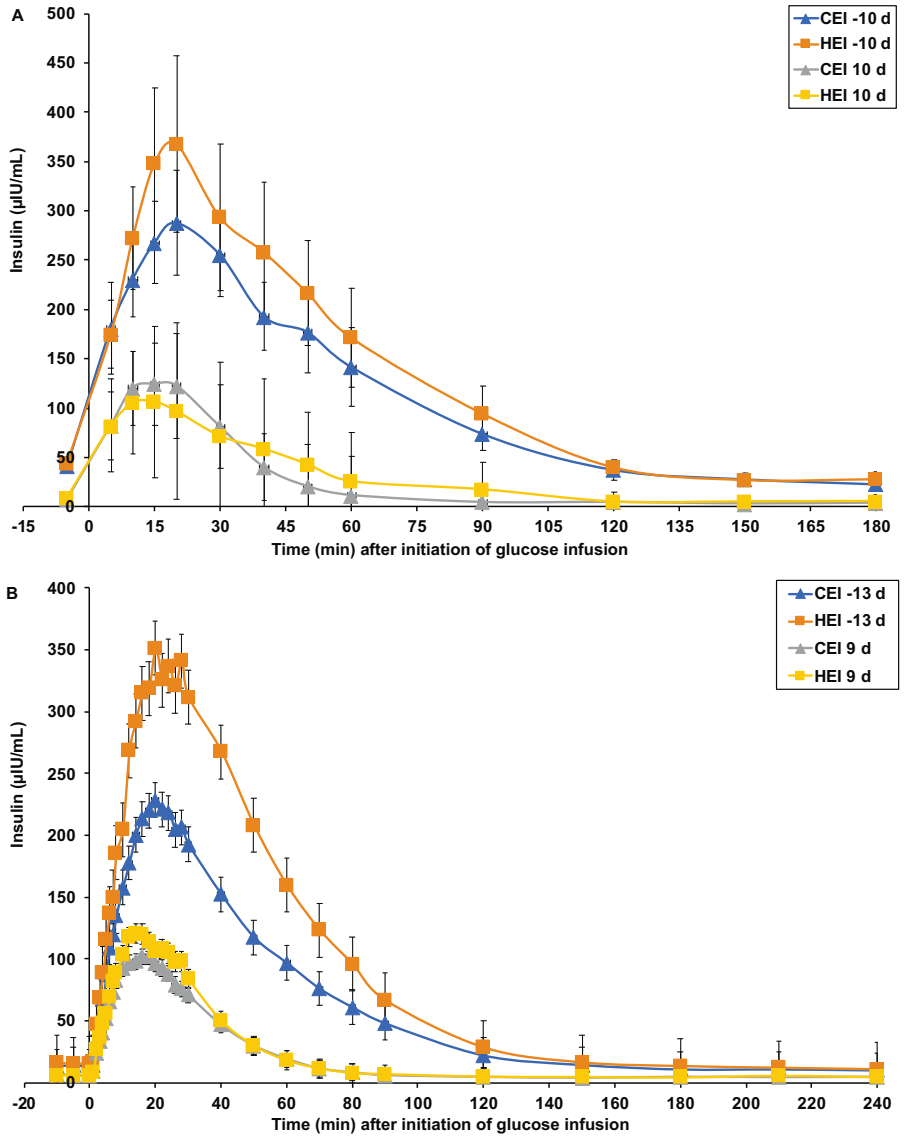

Figure 1 Insulin dynamics during IVGTT pre and postpartum in Exp. 2 (II; A) and in Exp. 3 (III; B). Treatments in Exp. 2 (II): CEI = 100% of ME requirements prepartum; HEI = 144% of ME requirements during weeks -6 to -4 followed by a gradual restriction of energy intake (119% of ME requirements) during the last 3 weeks of gestation. Treatments in Exp. 3. (III): CEI = 108% of ME requirements prepartum; HEI = 141% of ME requirements prepartum, *ad libitum* allowance.

4.4.4 NEFA DYNAMICS DURING THE IVGTT PREPARTUM

An impairment of insulin action at the level of adipose tissue may be a crucial contributor to metabolic imbalance of dairy cows accelerating the mobilisation of NEFA in overconditioned transition dairy cow (Holtenius et al., 2003; Holtenius and Holtenius, 2007; De Koster and Opsomer, 2013). As lipolysis is attenuated by insulin, any cause of insulin resistance in adipose tissue may further enhance mobilisation of NEFA (Pires et al., 2007b). The increased adipose tissue mass and a reduced insulin-mediated suppression of lipolysis associated with i.e. obesity may lead to lipid overflow in the circulation (Jocken and Blaak, 2008). This, in turn, induces additional insulin resistance as part of a vicious cycle, a phenomenon recognized in humans (Rosen and Spiegelman, 2006).

In dairy cows, it was recently shown *in vitro* that larger fat cells have higher basal lipolytic activity than smaller adipocytes (De Koster et al., 2016a). Also, the experimental elevation on plasma NEFA by lipid infusions induced an insulin resistance state in the AT as assessed by IVGTT and IC (Pires et al., 2007b, 2008). However, the decrement of NEFA by insulin is not necessarily associated with BCS and adiposity before calving (De Koster et al., 2015). Correspondingly, metabolic challenges during the transition period did not find any changes in pancreatic insulin secretion and peripheral insulin responsiveness in cows with a wide range of body condition (Weber et al., 2016).

Effect of induced higher plasma NEFA (I)

In Exp. 1 (I), during the IVGTT, an unchanged NEFA response was found between CON and lipid infusions when assessed by NEFA disposal (NEFA AUC and CR of NEFA). However, as the lipid infusions had decreased insulin secretion (I), lower insulin concentration was needed in lipid infused cows to elicit similar NEFA disposal than in CON cows. Similar effects were found in IC (I), because a tendency for lower basal insulin together with an unchanged insulin disposal during IC led to a more negative NEFA AUC after lipid than after CON infusions (I). These results alone suggest a more sensitive adipose tissue to insulin after lipid infusions. However, given that NEFA model index describing the rate of NEFA removal from the plasma pool (K_{FFA}) was decreased by approximately 50% after lipid infusions during the IVGTT, the findings point to an impaired NEFA clearance after induction of greater NEFA (I).

Effect of high vs. controlled energy intake prepartum (II-III)

In Exp. 2 (II) the prepartal energy did not affect insulin response during the IVGTT, but lower NEFA decrement was found in HEI than in CEI (Figure 2; A). This may be interpreted to represent a blunted NEFA response in HEI cows in response to a glucose bolus, and an indication of altered sensitivity to insulin in response to overfeeding of energy. In contrast, In Exp. 3. (III) the prepartal high energy intake induced a compensatory insulin secretion during

the IVGTT. Consequently, the HEI cows needed a higher insulin concentration (peak concentration and AUC of insulin) to elicit a similar NEFA response than CEI cows in prepartal IVGTT (Figure 2; B). In agreement with Exp. (II), these findings suggest that high prepartal energy intake affected insulin´s action on lipid metabolism and attenuated the adipose tissue response in HEI cows prepartum. Similarly, homeostatic challenges in early lactation cows of different strains showed that cows with greater basal NEFA had greater suppression of NEFA after IC than those with lower NEFA (Patton et al., 2009). These findings together with the current (II, III) suggest that basal NEFA is a factor contributing to the extent of the decrement of NEFA, as also shown in studies with transition dairy cows by Schoenberg et al. (2012) and De Koster et al. (2015).

The observed refractory adipose tissue response in HEI during prepartal IVGTT (II, III) as assessed by smaller NEFA decrement in response to similar insulin secretion (II) or to similar NEFA decrement in response to greater insulin secretion (III), indicate that prepartal energy level affected insulin sensitivity in adipose tissue before parturition. Although BCS prepartum did not differ between treatments in Exp. 2 (II, IV), the potential differences in adipose tissue accretion invisible to visual inspection may explain divergent NEFA responses. Correspondingly, cows that where losing high amounts of BW had more refractory adipose tissue to insulin both pre and postpartum (Zachut et al., 2013). By contrast, other recent research has not shown consistent effects of increased body fatness and high energy intake on inhibition of lipolysis by insulin or in insulin signalling in adipose tissue of transition dairy cows (De Koster et al., 2016b; Mann et al., 2016b; Jaakson et al., 2018). Recently, the metabolic responses to IVGTT were associated with the physiological state of the animal instead of the dietary treatment during different stages of extended lactation (Marett et al., 2015).

The discrepancies between the findings of the studies may suggest that when NEFA levels are experimentally induced by means of feed deprivation (Oikawa and Oetzel 2006; Pires et al., 2007b; Schoenberg et al., 2012) or by lipid infusions (I), the condition will rapidly affect a range of metabolic responses. Increased lipolysis and hypoglycemia may partially confound the treatment effects on metabolite and hormone concentration during both basal and challenged conditions.

Indeed, the previous attempts in assessment of effects of overconditioning or accumulation of adipose tissue and development of insulin resistance in regard to glucose and lipid metabolism lack consensus. For instance, insulin response of the glucose metabolism, but not that of fatty acid metabolism, had a negative association with excessive accumulation of adipose tissue in late pregnant dairy cows as assessed by HEC test (De Koster et al., 2015). As opposed to former, insulin sensitivity in adipose tissue of overconditioned cows with greater adipocytes was preserved *in vitro* (De Koster et al., 2016b). Recently, glucose transporter 4 protein synthesis in adipose tissue of overconditioned cows was reduced regardless of similar insulin signalling than

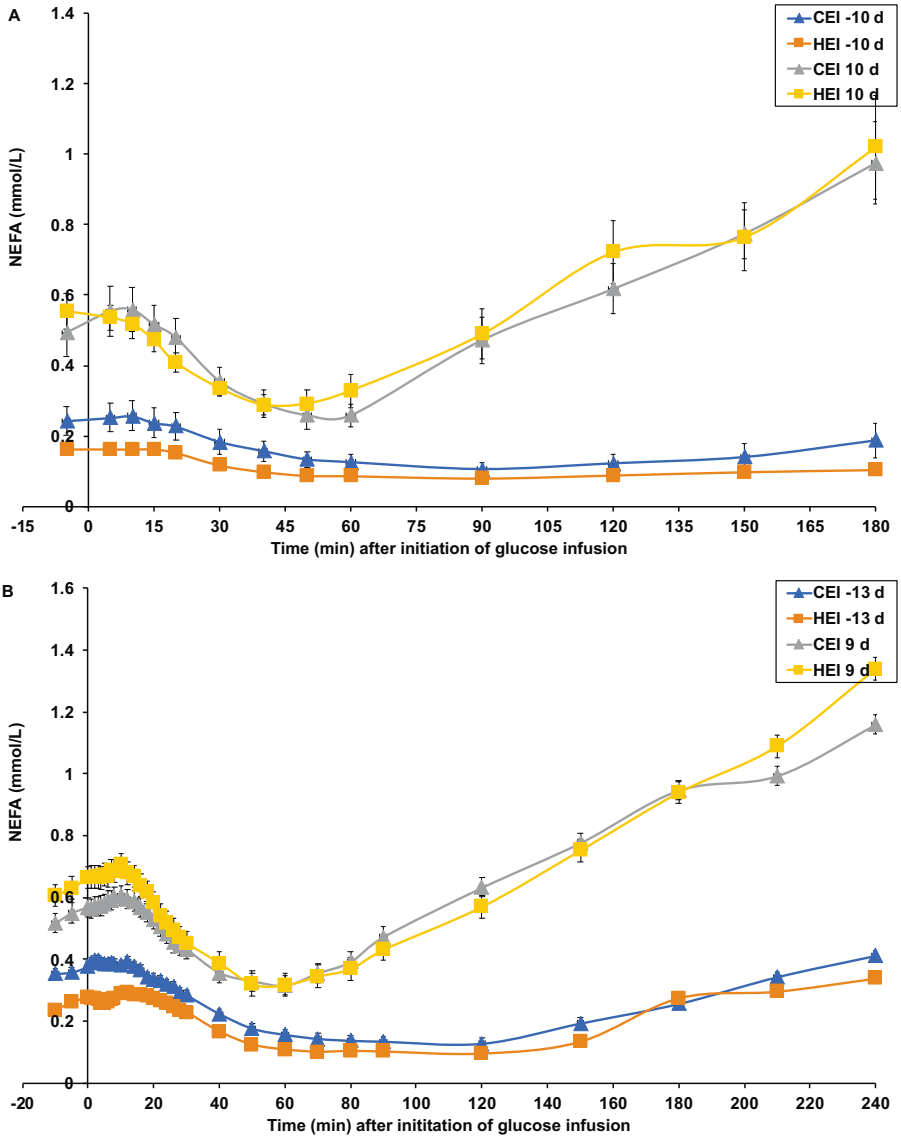

Figure 2 NEFA dynamics during IVGTT pre and postpartum in Exp. 2 (II; A) and Exp. 3 (III; B). Treatments in Exp. 2 (II): CEI = 100% of ME requirements prepartum; HEI = 144% of ME requirements during weeks -6 to -4 followed by a gradual restriction of energy intake (119% of ME requirements) during the last 3 weeks of gestation. Treatments in Exp. 3. (III): CEI = 108% of ME requirements prepartum; HEI = 141% of ME requirements prepartum, *ad libitum* allowance.

in thinner cows prepartum suggesting a more severe insulin resistance in adipose tissue of fatter cows (Jaakson et al., 2018). Conversely, the CUDP energy overfeeding did not exacerbate insulin resistance in adipose tissue, as assessed by changes in insulin signalling in subcutaneous adipose tissue (Ji et

al., 2012). In the former study, the insulin resistance in adipose tissue was more pronounced in late pregnancy, regardless of prepartal diet, while IS in adipose tissue was rapidly restored in early lactation. Further, in agreement with current results showing high interindividual variation in insulin responsiveness after a glucose bolus (II, III; Figure 1), a large number of studies have reported similar responses of IVGTT in dairy cows at various stages of lactation and pregnancy (De Koster et al., 2015; Marett et al., 2015; Mann et al., 2016a; De Koster et al., 2017; II, III). The most prominent reason for the high variance is the large reported variation in the initiation of the metabolic adaptations during the transition period (Jorritsma et al., 2003; Kessel et al., 2008; van Der Drift et al., 2012; Weber et al., 2013; II, III).

Taking into consideration the homeorhetic changes occurring during the CUDP, it is safe to assume that different mechanisms of action may control glucose and NEFA dynamics induced by insulin in late pregnancy and FODP (I) vs. CUDP (II, III). Indeed, earlier studies mimicking early lactation NEFA increment by abomasal infusions of lipids or by fasting or both in dry cows suggest, that different physiological phenomenon are responsible for the experimentally induced greater NEFA than what underlies negative EB occurring in early lactation (Pires et al., 2007b; Schoenberg et al., 2012). This further suggest that mechanisms affecting development of insulin resistance at the level of adipose tissue during artificial elevations of NEFA by lipid infusions differ from those that are involved in the development of insulin resistance in periparturient dairy cows (I, II, III).

During IVGTT in Exp. 1 (I), an initial increase in plasma NEFA concentration in association with higher basal NEFA induction was found (I). A similar observation was made from the visual inspection of the NEFA curves in Exp. 2 (II) and 3 (III) as NEFA response remained refractory to insulin during the first 10 min of the IVGTT both pre and postpartum, suggesting a delay in both insulin transport and signalling (Sumner et al., 2004). The 40% increased NEFA model-derived parameter for latency in HEI than in CEI prepartum reinforces that adipose tissue remained unresponsiveness to insulin (III). The latency is thought to result from the time required for insulin to pass to the interstitium (Jansson et al., 1993) to trigger the suppression of lipolysis (Bergman et al., 1979; Sumner et al., 2004; Boston and Moate, 2008). Taken together, these results suggest that both experimentally induced greater NEFA (I) and energy oversupply (III) and consequently greater BCS (III) may have at least partially delayed the time taken by the insulin to start exerting its antilipolytic effect prepartum.

4.4.5 NEFA MODEL ESTIMATES

Modelling approaches, analogous to the MM of glucose kinetics (Bergman et al., 1979), for quantification of the suppressive effect of insulin on NEFA disposal during IVGTT have been developed (Moate et al., 2007; Boston and Moate, 2008; Periwal et al., 2008). These methods measure the dynamic

response of NEFA after a glucose bolus and insulin stimulus (Periwal et al., 2008). The NEFA model (Boston and Moate, 2008) was used in the current series of experiments (I-III), and the reported values of NEFA dynamics (Table 5) agree with those reported earlier in dairy cows (Moate et al., 2007; Boston and Moate, 2008).

No statistical differences in NEFA model derived indices describing NEFA provision (S_{FFA}) and disposal (K_{FFA}) was found during the IVGTT (I-III). In Exp. 1 (I) the numerically 52% smaller rate of removal of NEFA from the plasma pool (K_{FFA}) during the lipid infusions than CON infusion is in alignment with increased basal NEFA levels (I). The finding indicate that experimentally induced greater NEFA levels deteriorated the removal of NEFA, as no treatment differences in the maximum rate of lipolysis (S_{FFA}) during IVGTT was found (Boston and Moate, 2008). Hence, the impaired clearance during TAL and CAM infusions was either due to lower uptake and oxidation of NEFA by lipid-utilizing tissues or due to decreased incorporation of FA into adipose tissue.

The effect of prepartal dietary energy on NEFA model derived indices was manifested by the greater change in initial NEFA concentration (FFA0) in HEI vs. CEI from pre to postpartal IVGTT (II), corresponding to observed differences in basal NEFA between the diets before the IVGTT. In addition, the greater NEFA model derived parameter for latency in HEI vs. CEI in Exp. 3 (II) suggests that adipose tissue remained unresponsive to insulin as a response to prepartal overfeeding of energy.

A number of reasons may underlie the discrepancies between the observed NEFA responses assessed by calculated values describing the NEFA dynamics (nadir, AUC, CR, and decrement of NEFA) and those obtained from the NEFA model (I-III). Firstly, in dairy cows, the NEFA model (Boston and Moate, 2008) which is based on non-insulin-modified IVGTT have been used in only few studies (Moate et al., 2007; Roche et al., 2008; Boston et al., 2008) before incorporated into the experiments included in this thesis (I, II, III). Secondly, the use of the model in such low number of studies in dairy cows warrant future research in order to validate the model parameters against other existing models measuring insulin sensitivity of adipose tissue in these species. Thirdly, in dairy cows in early lactation, the basal glucose, and insulin levels are low due to redirection of glucose into the mammary gland, which may affect the interpretation of NEFA dynamics. The hypoglycemia following insulin response after a glucose bolus, and the ensuing counter-regulatory response, may per se induce hormonal changes which alter NEFA dynamics during the IVGTT (Thomaseth et al., 2014).

The developed NEFA models have been suggested being more reliable in detecting differences in adipose tissue responses to insulin than what is revealed during constant insulin infusion during clamps (Periwal et al., 2008), provided the counter-regulation is prevented. This would be possible if the NEFA model were incorporated into insulin modified IVGTT, together with administering also exogenous glucose during the test (Thomaseth et al., 2014).

The administration of exogenous insulin at 20 min after the glucose infusion extends the assessment of glucose dynamics to individuals with impaired insulin sensitivity and/or secretion, while the glucose infusion avoids a hypoglycemia-induced counter-regulatory response, which itself can markedly alter lipolysis (Thomaseth et al., 2014). This procedure would benefit the assessment of insulin resistance in dairy cows during the early lactation, when both insulin and glucose levels are low.

4.4.6 POSTPARTAL EFFECTS DURING THE IVGTT

The effect of prepartal energy intake on postpartal glucose, insulin and NEFA dynamics were mostly absent in current experiments (II, III). A consistent NEFA rebound was observed across the treatments and cows in early lactation (II, III; Figure 2). This was evidenced as an increase of NEFA levels above the initial basal level after the decrement and the steady incline of NEFA at around 60 min of IVGTT. The strong rebound in postpartal IVGTT indicates resumption of lipolysis potentially because of a surge of epinephrine and an increase in growth hormone concentration (Sumner et al., 2004; Roche et al., 2008) occurring as a response to hypoglycemia after insulin concentrations are already declining (Figure 3). These adjustments are reportedly occurring around the time the NEFA rebound is initiated (I-III), as shown after a glucose bolus during IVGTT in dairy cows (Roche et al., 2008). Also, other hormones are secreted in response to glucose bolus, such as leptin, ghrelin and glucagon (Roche et al., 2008). Especially during the early lactation, the tissue sensitivity to stimulatory effect of lipolytic hormones is increased (Drackley et al., 2005; Kokkonen et al., 2005; Roche et al., 2013). Not only low insulin concentration, but also low IGF-1 and elevated growth hormone levels (Hart., et al., 1978; Zhao et al., 1996; Rhoads et al., 2008) characterize early lactation. These changes may partially explain the observed NEFA rebound after parturition across the cows (II, III).

In Exp. 2 (II) the adipose tissue of HEI was more sensitive to insulin after parturition than that of CEI, as assessed by a greater decrement of NEFA concentration after glucose infusion in IVGTT, without any differences in insulin concentrations during the challenge. Across the animals, the CR of NEFA was unchanged from pre to postpartum (II), suggesting that increased lipolysis was the primary reason for the observed difference. This more sensitive adipose tissue in HEI postpartum was unexpected, and reasons for the greater NEFA response may be several. Firstly, the individual variation in the sensitivity to the changes in the lipolytic hormones may play a role in observed results. Secondly, the possible differences in visceral fat accumulation, which were beyond the determination methods of current studies, may contribute to postpartal NEFA response. Indeed, in dairy cows the visceral adipose tissue is more readily mobilized than subcutaneous adipose tissue shortly after parturition (Akter et al., 2011) and there is evidence to support that visceral adipose tissue accumulation is varied between

individual animal and adipose tissue site (Hostens et al., 2012; Drackley et al., 2014).

Further, the postpartal findings in Exp. 2 (II) represent an apparent conflict against the general assumptions in regard to adipose tissue energy partitioning and the role of insulin in early lactating dairy cow. It may be speculated that the gradual restriction of DMI during the CUDP in HEI (Exp. 2; II) may have diminished any further effects of overfeeding on body mobilisation, as the feed intake decreased by around 30% during the last 4 weeks. On the other hand, studies on MS-based diets showed beneficial effects of FODP energy restriction on cows in early lactation metabolism, while no beneficial effects were seen when energy intake was restricted in CUDP (Dann et al., 2006; Cardoso et al., 2013). Given the adipose tissue is a spare energy store which must rapidly respond to altered needs of energy in early lactation, it would be of value that insulin sensitivity of adipose tissue is well preserved. An insulin sensitive adipose tissue adjusts to nutrient deficiency assisting the animal in coping with the massive stress caused by the onset of lactation.

Across the cows (II, III) NEFA decrement was strongly correlated with basal NEFA concentrations (II, III) in agreement with results in dry cows and cows in early lactation (Patton et al., 2009; Schoenberg et al., 2012). In humans the inhibitory action of insulin on adipose tissue lipolysis is dependent on the prevailing lipolytic activity, such that the antilipolytic effect of the hormone is more pronounced when the rate of lipolysis is augmented, probably due to increased insulin receptor and signal transduction activity (Zierath et al., 1998). Similarly, in dairy cows the antilipolytic action of insulin may be most evident when plasma NEFA are increased (Sechen et al., 1999), supporting the current findings (I-III). Thus, it may be relevant to speculate that the lipolytic state of the animal dictates the extent by which insulin elicits its effect on lipolysis inhibition. Moreover, individual differences in the regulation of lipolysis may affect the NEFA responses during the IVGTT, mediated by altered responses and sensitivity to catecholamines (Chilliard et al., 2000; Drackley et al., 2005). Similarly, the increment of adiposity increases lipolytic response to adrenaline in dairy cows in the dry period (Theilgaard et al., 2002). It was recently shown *in vitro* that larger fat cells have higher basal lipolytic activity than smaller adipocytes (De Koster et al., 2016a), albeit others found no relationship between adipocyte size and lipolytic activity (Pike and Roberts; 1984). The current (I-III) and earlier results may suggest that the extent of NEFA decrement under stimulated conditions is only partially a product of the direct effect of insulin response (the insulin AUC) after the glucose bolus. The decrement may also be partially mediated by the secondary effect of insulin and lipolytic agents on basal lipolysis before IVGTT. In all, the data suggest that although insulin is partly responsible for the basal variation of NEFA and inhibits lipolysis during the postpartal IVGTT with low basal insulin concentrations, other mechanisms also mediate adipose tissue response to glucose stimulus.

4.4.7 PRE VS. POSTPARTAL EFFECTS DURING THE IVGTT

In Exp. 2 (II) where the comparison between prepartal and postpartal responses to insulin and glucose were evaluated, the calculated values describing dynamic changes in plasma glucose, insulin and NEFA concentrations followed a typical course of action in transition dairy cow. Across the animals (II), the basal plasma glucose in postpartal IVGTT decreased by 30% when compared with prepartal IVGTT (4.4 vs. 3.1 mmol/L). Similarly, peak value of glucose decreased by 20% (17.0 vs. 15.5 mmol/L) and AUC by 30 % (441 vs. 308 mmol/L x 180 min) from prepartal to postpartal IVGTT across treatments. The clearance rate of glucose increased by around 40% after calving (1.7 vs. 2.7%/min, in agreement with Holtenius et al., 2003, reporting numerically higher glucose CR in GS-based diets. Other studies investigating glucose and insulin dynamics in early lactation versus late pregnancy have reported either increased clearance of glucose after parturition (Bossaert et al., 2008; Mann et al., 2016b) or no alterations in clearance of glucose during the transition period (Grünberg et al., 2011; Zachut et al., 2013) in response to varied glucose boluses. The differences in the length of the fasting period before the challenges may have an impact on the varied findings on glucose and insulin dynamics, given that not only insulin secretion but also glucose clearance is reduced during fasting (Oikawa and Oetzel, 2006; Schoenberg et al., 2012). Additionally, in Exp. 2 (II) the use of glucose by tissues other than mammary gland (based on the calculated glucose use per unit of insulin, see page 27) was 66% higher after calving than during late pregnancy (20 vs. 12 mg/μIU per millilitre × 30 min). This suggest that glucose uptake by insulin-sensitive tissues may be proportionally greater during the early stages of the challenge (0 to 30 min). Because this effect was abolished after 60 min of IVGTT, it was concluded that tissue responsiveness to insulin is enhanced after parturition under stimulated conditions (i.e. in high insulin concentrations in early stages of IVGTT). Indeed, a recent study demonstrated that insulin response to glucose load was greater at 150 days in milk (DIM) than at 50 or 100 DIM. This may be interpreted to suggest improvement of insulin sensitivity in early vs. later stage of lactation, because insulin AUC was greater at 150 than at 50 DIM and glucose CR and AUC were unaltered (Oliveira et al., 2016).

Altogether the former findings and the observed changes in other calculated values during the IVGTT pre vs. post (II) suggest that the low insulin concentration postpartum is most likely the main factor regulating glucose utilization by peripheral tissues during early lactation. In line with former, the insulin response to glucose load across the animals decreased after parturition. In addition to the decline in basal insulin, peak insulin (357 vs. 129 μIU/mL) and the AUC180 of insulin (13,510 vs. 3,673 μIU/mL x 180 min) decreased after calving (II). The increment of CR of insulin (0.73 vs. 2.4%/min, pre and postpartum, respectively) is in agreement with Bossaert et al., (2008) and Mann et al. (2016b).

As reported earlier, the pancreatic responsiveness to insulinotropic agents are greatly depressed during early lactation (Lomax et al., 1979). The approximately 2 to 3-fold lower postpartal than prepartal insulin response (II, III), evaluated by peak insulin, and insulin AUC (II) agree with several published metabolic challenge data in dairy cows with wide range of body mobilisation (Holtenius et al., 2003; Zachut et al., 2013; Bossaert et al., 2008; Mann et al., 2016b; Weber et al., 2016; Jaakson et al., 2018). It seems that the low insulin level is the most established adjustment in the early lactation period, and the main driver regulating glucose use by peripheral tissues. In agreement with the comparisons between the time points across the treatments (II) greater prepartal insulin in response to overfeeding was found, while lower insulin levels were shown postpartum (Mann et al., 2016a, Jaakson et al., 2018; III). The low circulating insulin is a result of lower response to a similar secretory stimulus (Bossaert et al., 2008; Kerestes et al., 2009; Zachut et al., 2013; II, III) and increased clearance of insulin (Mann et al., 2016a; II, III). The naturally occurring decrement of basal insulin level after parturition support the lipolytic state indicated by greater plasma NEFA and enhance hepatic gluconeogenesis to cope for the energy deficiency (Bell, 1995; Bauman and Currie, 1980; Ingvartsen and Andersen, 2000). Additionally, the higher plasma NEFA concentrations across the cows post vs. prepartum (II, III) may have partially inhibited insulin secretion in early lactation due to FA negative impact on pancreatic β-cell function (Zhou and Grill, 1994; Xiao et al., 2006; Giacca et al., 2011; I).

The NEFA response to the glucose load across the animals was unchanged as assessed by NEFA clearance between pre and postpartal IVGTT, which is unexpected (II). However, a decreased CR of NEFA and higher nadir of NEFA during the postpartal IC together with greater NEFA model parameters describing NEFA dynamics in IVGTT postpartum (II) reinforce the increment of the lipolytic activity in early lactation (Figure 2). The pronounced decrease in insulin concentration towards the onset of lactation (II, III), together with the higher basal NEFA and nadir of NEFA postpartum than prepartum (II, III), reflect the lipolytic state of early lactation. Moreover, the NEFA rebound, the period when NEFA increases back to baseline from the nadir concentration (Moate et al., 2007; Boston and Moate, 2008) was prolonged prepartum, as the NEFA concentrations across the cows did not return to basal levels during the prepartal IVGTT (II, III). The period represents the action of counterregulatory hormones (Sumner et al., 2004; Thomaseth et al., 2014) and suggests a resumption of lipolysis, as a result of declined insulin concentrations and subsequent increase in epinephrine and growth hormone concentration during an IVGTT (Sumner et al., 2004; Roche et al., 2009). In contrast, after parturition all cows rebounded to suprabasal NEFA levels which were preserved until the end of the challenge. The greater rebound after than before parturition across cows in Exp. 2 (II) and Exp. 3 (III) confirm the increased adipose tissue sensitivity to lipolytic stimulation (Bauman and Currie, 1980; Bell, 1995), mediated by growth hormone and catecholamines in

early lactation (McNamara, 1997; Bauman, 2000; Chilliard et al., 2000; Drackley et al., 2005). The abrupt shift from anabolic to catabolic activities at the onset of lactation is not only supported by increased sensitivity to lipolytic stimulation but also by diminished maximal inhibition of lipolysis (Bauman and Currie, 1980; Bell, 1995).

In conclusion, the effects of prepartal high-energy intake affected mainly prepartal peripheral insulin resistance status as assessed by deteriorated adipose tissue response to insulin (II, III) and by compensatory insulin secretion to preserve glucose tolerance (III). complementing the findings of the lipidomics data on the same animals (Qin et al., 2017, 2018). The results suggest that peripheral insulin sensitivity of dairy cows is not profoundly affected by the prepartal dietary treatment during the transition period regardless of the degree of body fat mobilisation, in agreement with recent published data (Ji et al., 2012; De Koster et al., 2016b; Mann et al., 2016b; Weber et al., 2016).

4.4.8 MINIMAL MODEL ESTIMATES

The minimal model approach incorporated into traditional non-insulin modified IVGTT (without exogenous insulin at +20 min) is widely used in human and animal models to study the different pathological stages of glucose tolerance and insulin sensitivity (Ayala et al., 2010; Dube et al., 2013). This dynamic model is also applied in investigations of variety of diabetic conditions, as reviewed e.g. by Ferrannini and Mari (1998) and DeFronzo et al. (2015). The IVGTT with MM approach are commonly used for longitudinal studies also when modelling the development of human obesity and type 2 diabetes in canines (Mittelman et al., 2000; Kim et al., 2007; Stefanovski et al., 2011) while in horses MM is an established method for assessment of obesity induced insulin resistance (Hoffman et al., 2003; Frank and Tadros, 2014).

Glucose is produced and released by the liver in a non-stimulated, basal state. After an intravenous glucose injection (Figure 3), the levels of plasma glucose rapidly increase to a peak, where after the glucose will be disposed in the peripheral tissues by two ways. The minimal model produces indices that describe the dynamic changes during the IVGTT. The initial glucose disposal after the glucose injection represents the glucose effectiveness (Sg). Glucose effectiveness is the capacity of the glucose to mediate its own disposal under basal insulin. The insulin mediated glucose disposal is characterized by the acute insulin response (AIRg), which represents the production of insulin by the pancreas during the first 10 minutes after glucose injection. The first-phase insulin response is a useful measure of β-cell function (Kahn et al., 1993). The disposition index (DI) is defined as the product of AIRg and SI. The DI characterizes the hyperbolic relationship between resistance and secretion of insulin; metabolic compensation is maintained against decreased SI only if insulin secretion increases (Kahn et al., 1993; Bergman, 2007). The DI is in

humans genetically determined (Palmer et al., 2006; Bergman, 2007), and is the most accurate physiological measure for the quantification of the compensation for the pancreatic insulin release in response to changes in insulin resistance of peripheral tissues (Bergman, 2002; Stefanovski et al., 2011).

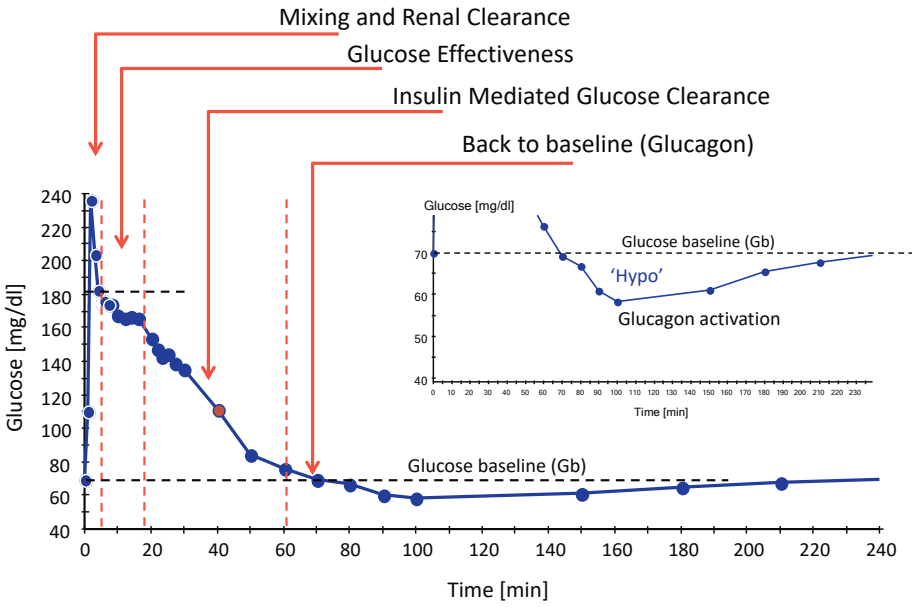

Figure 3 Factors affecting glucose dynamics during IVGTT.

In dairy cows it is not established to use the MM approach in association with IVGTT. Although the major energy substrate (the ruminally derived VFA of the cows in a fed state) differs from that of humans, previous and present investigations (I-III) indicate that glucose MM parameters of lactating dairy cows are similar in magnitude to those reported for humans (Bergman, 2002, 2007; Boston et al., 2008) and for other mammalian species such as dogs, horses and sheep (Mittelman et al., 2000; Hoffman et al., 2003; Williams et al., 2004). This implies that, in ruminants, the MM parameters are equally related to the central processes of glucose metabolism and its control by insulin (Moate et al., 2007). Previously, studies comparing MM derived SI and that of HEC reported a good agreement between these two indices (Stanley et al., 2005; De Koster et al., 2016a). Indeed, the MM derived index of SI was considered to represent a more reliable estimate of the overall insulin sensitivity than AUC of glucose (De Koster et al., 2016a). In humans the MM estimates have shown very strong correlations with values derived from gold standard test (the HEC test; Bergman et al., 1987; Saad et al., 1994). It has also been confirmed that the parameters SI and Sg derived from

unmodified (non-insulin assisted) IVGTT are concordant with those derived from clamp techniques in dogs and humans ($r > 0.70$; Finegood et al., 1984; Bergman et al., 1987; Steil et al., 2004). In cattle, Stanley (2005) concluded that both tests are adequate for measuring differences of insulin sensitivity between treatments. The HEC test have recently been suggested to represent the gold standard for assessment of insulin resistance in ruminant research (De Koster and Opsomer, 2013; De Koster et al., 2015; 2016a), similarly to humans (Ferrannini and Mari, 1998; Muniyappa et al., 2008).

Stanley (2005) observed that estimates of insulin sensitivity derived from MM and HEC were positively correlated in calves but not in lactating cows. When SI was compared with 3-months and 6-month old calves the SI was decreased along with increasing age (Table 6). Indeed, these results suggest that mature cattle are more insulin resistant than calves, as assessed by MM estimates of SI. As the cow mature, the glucose absorbed from the duodenum is negligible, and the supply of glucose is almost exclusive dependent on hepatic gluconeogenesis. Moreover, the mature dairy cow is either in pregnant or lactating state, and during lactation insulin-independent glucose uptake by the mammary gland may constitute 60 to 90% of the glucose disposal (Rose et al., 1997; Bauman and Currie, 1980; De Koster and Opsomer, 2013). Consequently, it has been argued that due to these specialities in glucose metabolism of the lactating dairy cow, comparison of insulin resistance state in late pregnancy versus early lactation may introduce bias to interpretations, and further research is warranted in this area. Thus, in the current research (I-III) the metabolic dynamics during the IVGTT were incorporated into MM analysis. Beside MM derived indexes, additional calculations of inter-relations between insulin and glucose dynamics during the challenge in Exp. 2 (II) were conducted, in order to verify that the comparison between pre- and postpartal responses of individual cow to exogenous glucose during the IVGTT are relevant.

The estimated prepartal SI values (Table 6) of the current studies (I-III) were within the range of values reported in the dry pregnant cows (De Koster et al., 2016a) or lower than the reported values in De Koster et al. (2017). The postpartal values were similar to or lower than those of lactating cows (Stanley, 2005; Moate et al., 2007; Marett et al., 2015; De Koster et al., 2017; II, III) and lower than those reported in calves (Bunting et al., 2000). The greater prepartal values of SI in Exp. 1. (I) than those in Exp. 2. (II) and Exp. 3 (III) are most probably due to differences in the parameter estimation, as in study I the estimations are based on data gathered from earlier population studies (Boston, R., personal communication), while in II and III a combined population consisting of experimental animals from Exp. 1 – 3 were used. Also, the stage of dry period (FODP vs. CUDP) most likely affect in the observed varied responses, indicated by statistical significances in most of the calculated parameters relative to days to parturition (II).

Table 6. *Estimates of insulin sensitivity as assessed by minimal model in dairy cows*

Subject	n[1]	SI[2]	Sg[3]	AIRg[4]	DI[5]	Reference
Ayrshire dairy cows at -31 ± 12 d to parturition CON infusion	6	1.03	0.01	449	447	I[1]
Ayrshire dairy cows at -31 ± 12 d to parturition TAL infusion	6	0.73	0.01	435	299	
Ayrshire dairy cows at -31 ± 12 d to parturition CAM infusion	6	1.39	0.01	380	489	
Ayrshire dairy cows at -10 ± 5 d to parturition CEI diet	15	0.69	0.01	1099	728	II[2]
Ayrshire dairy cows at -10 ± 5 d to parturition HEI diet	14	0.61	0.02	1217	500	
Ayrshire dairy cows at 10 ± 1 d to parturition CEI diet	15	3.40	0.01	640	2060	
Ayrshire dairy cows at 10 ± 1 d to parturition HEI diet	14	4.20	0.01	605	1652	
Ayrshire dairy cows at -13 ± 5 d to parturition CEI diet	16	0.67	0.02	695	227	III[3]
Ayrshire dairy cows at -13 ± 5 d to parturition HEI diet	16	0.23	0.03	981	241	
Ayrshire dairy cows at 9 ± 1 d to parturition CEI diet	16	1.74	0.03	456	825	
Ayrshire dairy cows at 9 ± 1 d to parturition HEI diet	16	2.28	0.03	512	1065	
Holstein dairy cows at -18 ± 2 d to parturition (2.5 < BCS ≤ 3.5)	5	1.29	—	1505	—	De Koster et al., 2016a &
Holstein dairy cows at -18 ± 2 d to parturition (3.5 < BCS ≤ 5.0)	5	0.77	—	2799	—	Bogaert et al., 2018
Dry, non-pregnant Holstein heifers	5	0.95	0.02	—	—	De Koster et al., 2017
Dry, pregnant Holstein heifers at -10 ± 2 d to parturition	5	3.99	0.02	—	—	
Lactating primiparous Holstein dairy cows at 12 ± 1 d	5	3.83	0.03	—	—	
Holstein dairy cows at 21 ± 3.5 d to parturition	5	12.9	0.02	—	—	Moate et al., 2007
Holstein dairy cows at -1 wk to parturition	41	1.88	0.02	265	—	Stanley, 2005
Holstein dairy cows at 1 wk to parturition	41	3.45	0.02	184	—	
Holstein dairy calves at the age of 3 weeks	3	18.1	0.02	—	—	Stanley et al., 2002
Holstein dairy calves at the age of 6 weeks	3	10.5	0.02	—	—	
Holstein dairy cows at +100 ± 9 d parturition + 1 kg grain/d	12	4.20	0.02	308	985	Marett et al., 2015
Holstein dairy cows at +250 ± 9 d to parturition + 1 kg grain/d	12	6.90	0.02	134	756	
Holstein dairy cows at +100 ± 9 d parturition + 6 kg grain/d	12	6.80	0.02	185	1162	
Holstein dairy cows at +250 ± 9 d parturition + 6 kg grain/d	12	9.30	0.02	139	1019	

[1] Exp.1 (I): CON = abomasal infusion of water (98 h); TAL = abomasal infusion of tallow (98 h); CAM = abomasal infusion of camelina oil (98 h).
[2] Exp. 2 (II): CEI = 100% of ME requirements prepartum; HEI = 144% of ME requirements during weeks -6 to -4 followed by a gradual restriction of energy intake (119% of ME requirements) during the last 3 weeks of gestation.
[3] Exp. 3. (III): CEI = 108% of ME requirements prepartum; HEI = 141% of ME requirements prepartum, *ad libitum* allowance.

Effect of induction of higher NEFA levels (I) on MM estimates

Compared with TAL, infusion of CAM increased insulin sensitivity index (SI) by 90% (1.39 vs. 0.73 x 10^{-4} min^{-1}/μIU/mL). However, compared to CON, the SI was not affected by lipid treatments, mainly because of the large range in SI between different lipid sources. The numerically lower acute insulin response (AIRg) after CAM infusion indicates a decreased need for an acute insulin burst after glucose load because of tendency for increased SI contributed to a significantly greater DI in CAM vs. TAL (39%; 489 vs. 399 ± 64). Given that pancreatic β-cells are capable of upregulating insulin secretion in response to insulin resistance ("compensatory insulin secretion") and DI represents the extent of this action (Bergman, 2007), the DI value should have been greater in TAL than in CAM in order to show compensation for the lower SI (I). The results point to an insulin sensitizing effect of CAM vs TAL and suggest that CAM-infused cows were able to more efficiently compensate for insulin resistance induced by higher NEFA by increasing their β-cell responsivity. The finding that numerically lower insulin concentrations where needed in IC in CAM vs. TAL to elicit a similar clearance of glucose, reinforce the suggestion that CAM may have improved the peripheral insulin sensitivity when compared with TAL (I).

As already stated earlier, the significantly higher DMI between cows on water vs. lipid treatments (I) may have partially masked the effect of lipid infusions vs. water infusion, because of a higher DMI and propionate production and hence improved glucogenic status of the cows. In agreement with current findings (I), ingestion of palm oil supplement, rich in SFA induced insulin resistance compared with other fat sources, evidenced by decreased SI, which could not be compensated by adequate β-cell response in humans (Xiao et al., 2006). Interestingly, the authors suggested that reduced clearance of insulin may compensate for decreased insulin secretion in response to PUFA ingestion to preserve glucose tolerance. Saturated C16:0 has also been shown to reduce the proliferative capacity of β-cells in rodents and induced β-cell death mainly by apoptosis (Maedler et al., 2001). Palmitic (C16:0), palmitoleic (C16:1), stearic (C18:0), and oleic (C18:1) acids are the major fatty acids in the adipose tissue of dairy cows (Gillis et al., 1978; Smith et al., 1978; Rukkwamsuk et al., 1998) and in TAL infusate (I).

Effect of prepartal energy intake on MM estimates

The dry period energy intake had minor effects on MM estimates (II, III). Also, in nondiabetic subjects with a range of body adiposity, the AIRg demonstrated a broad range of insulin sensitivity and β cell function (Kahn et al., 1993), which may explain the non-existent differences between treatments. The only significant difference between dietary treatments in MM estimates was the 40% greater AIRg in HEI than in CEI during the prepartal IVGTT (III; 981 vs. 695 μIU/mL per min, in HEI vs. CEI, respectively). This is consistent with the observed greater insulin AUC of HEI (III), indicating that the compensatory insulin response to altered insulin sensitivity (numerically lower SI in HEI vs.

CEI), was successful in preserving the glucose tolerance. In contrast to current results, Marett et al. (2015) found a tendency for lower AIRg value in early lactation on 6 kg/d grain fed vs. 1 kg/d grain fed cows (185 vs. 308 mU/L per min, respectively) with similar SI values which may point to a compromised pancreatic insulin responsiveness to glucose load as a response to an approximate 28% greater ME intake. Accordingly, in Exp. 2 (II) the moderate over-consumption of energy in the dry period (II) tended to decrease the DI by 30% and 20% in HEI vs. CEI, pre and postpartum, respectively. This may insinuate compromised compensation for decrement of tissue sensitivity to insulin. However, the absence of dietary effects on other calculated values of glucose and insulin dynamics (II) suggest that moderate overfeeding did not affect insulin sensitivity of glucose metabolism in late pregnant dry cows in agreement with (Schoenberg and Overton, 2011). The primary reason for the lack of major effects of energy overfeeding on glucose and insulin dynamics (II) seems to be that increased energy supply in terms of GS feeding did not affect BW or BCS changes during the close-up dry period or the lipid mobilisation after calving (II). As already discussed in this thesis, impaired insulin action has been closely linked to overconditioning in transition dairy cows (Holtenius et al., 2003; De Koster et al., 2015; Jaakson et al., 2018).

Hyperbolic relationship between insulin secretion (AIRg) and insulin sensitivity SI

In healthy individuals with normal glucose tolerance, a reduction in insulin sensitivity in response to any environmental stimuli (such as obesity) is normally accompanied by a compensatory up-regulation of insulin secretion in response to glucose (Bergman, 1989; Kahn et al., 1993). Further, the MM derived DI, which is the product of AIRg and SI, characterizes the relationship between insulin sensitivity and insulin secretion and is reported to represent an approximate hyperbola in nature (Figure 4). The relationship between insulin sensitivity and plasma insulin levels in healthy individuals is reciprocal and nonlinear in nature (Kahn et al., 1993, 2006). Simplified, the former means that DI should remain constant for individuals with same degree of glucose tolerance (Kahn et al., 1993). The DI is one of the best predictors of progression to diabetes in humans (Alonso et al., 2012).

The hyperbolic relationship (II, III) was confirmed for the first time in dairy cows by these studies and is in agreement with the findings in human studies (Kahn et al., 1993; Bergman, 2007). As visualized in Figure 4, the sensitivity and secretion of insulin should vary according to the physiological state (e.g. pregnant and lactating) such that the values of DI stay on the hyperbola. Any deviation to the left and below are indications of deteriorated compensation for increased insulin resistance. DI is thought to characterize the ability of pancreatic β-cells to compensate for the variations in insulin sensitivity to preserve glucose tolerance (Kahn et al., 1993; Ahrén and Pacini, 2004; Bergman, 2002; 2007). The hyperbolic relationship suggests that alterations in environmental factors affecting insulin sensitivity for instance in response

to obesity (Mittelman et al., 2000) or overconsumption of energy (III), will be compensated by an increase in insulin secretion in response to glucose (Bergman, 1989; Kahn et al., 1993; Mittelman et al., 2000; III). The mechanism underlying the compensation may be related to alterations in both secretion and clearance of insulin. Research in overconditioned canines and equines showed that deterioration of peripheral insulin sensitivity was initially compensated by increased insulin secretion (Mittelman et al., 2000; Kim et al., 2011; Frank and Tadros, 2014), and subsequently by decreased hepatic insulin clearance (Mittelman et al., 2000; Kim et al., 2011).

The hyperbolic relationship of insulin sensitivity and insulin secretion reflects tendency to maintain plasma glucose concentration within limits by coordinated function of several organs (pancreas, liver, and adipose and muscle tissue) (Ionut et al., 2013). This means that especially in frank insulin resistance stages (i.e. when SI < 2.0 - 3.0) as typically found in association with obesity (Bergman et al., 1979; Bergman, 1989, 2002) small changes in insulin sensitivity are associated with large changes in fasting insulin. In these conditions, the basal insulin level has a negative correlation with SI. In comparison, in normal, glucose tolerant individuals with a wide range of SI (3.0 < SI < 10.0) a similar significant correlation is not necessarily observed (Shervin et al., 1974; Bergman et al., 1979). Given the low values of SI (range from 0.23 to 1.39) across the animals and experiments prepartum (I-III), the former may indicate that in ruminants the basal insulin level may be a good indicator of insulin resistance state with low SI values (I-III). This interplay between insulin sensitivity of peripheral tissues and insulin secretion facilitates a better adaptation for early-lactation dairy cows to changes in dietary composition and energy intake.

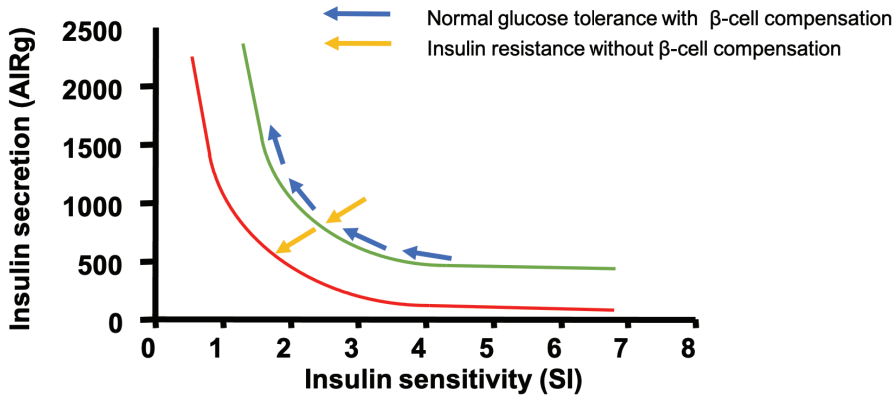

Figure 4 The hyperbolic law of glucose tolerance (adapted from Bergman et al., 2002).

The values of SI reported in this thesis (I-III) are in line with other earlier reports in cows (Table 6; Stanley, 2005; Moate et al., 2007; Marett et al., 2015; De Koster et al., 2016a, 2017; Bogaert et al., 2018), and reflect the development of insulin resistance and the alterations in physiological and metabolic status in transition dairy cows. Also, the MM derived value of glucose effectiveness Sg (I-III) agree with reported values in other mammalian species (Mittelman et al., 2000; Williams et al., 2004; Hoffmann et al., 2003) and those of humans (Beard et al., 1986; Bergman, 1989). The findings give further evidence of the reproducibility of the MM indices in dairy cows. It can be concluded that although glucose metabolism is clearly less responsive to insulin in ruminants than in other species, values for insulin sensitivity are comparable between species, in agreement with early studies on small ruminants (Bergman et al., 1989; Petterson et al., 1993). Indeed, the metabolic milieu of pregnant dairy cow resembles that of normal human pregnancy, in which insulin resistance and hyperinsulinemia are associated with a paradoxical decline in fasting glycemia, suggesting in agreement with current results (II, III) that glucose itself may not be the only signal that can upregulate pancreatic sensitivity in insulin-resistant states (Bergman, 2002).

4.4.9 PRE VS. POSTPARTAL EFFECTS ON MINIMAL MODEL INDICES

Consistently, across the dietary treatments (II, III), the MM derived values of SI were approximately 6-fold greater postpartum than prepartum (II) while similar numerical changes were observed in Exp. 3. (III) during the transition period. When the relationship of SI and AIRg is visualized (Figure 5; A and B), the changes in DI during transition period point to an improvement in overall sensitivity of tissues to insulin in early lactation (II, III). Besides SI values, only a very limited number of studies have reported other MM derived values in ruminant species.

The left and upward shift of the DI values of HEI (III) before parturition underpin that the compensatory insulin secretion to match the decrease in insulin sensitivity was sufficient in these animals. In contrast, a similar compensatory effect was not evident in experiment 2 (II) when a more moderate over-consumption of energy prepartum did not affect carbohydrate metabolism of the cows. Taken into a consideration that the DI reflects the ability of the pancreatic β-cells to compensate for increased insulin resistance of peripheral tissues in regard to glucose uptake (Bergman, 1989), the very low prepartal values of DI across the treatments point to an insulin-insensitive pancreas in response to glucose and to an overall lower compensation for decreased insulin sensitivity in all animals, as indicated also by very low SI values, in agreement with earlier studies (Kräft, 2004; Stanley, 2005; De Koster et al., 2016a). The improvement of SI values across the animals in early lactation (II, III), in alignment with earlier studies (Kräft, 2004; Stanley et al., 2005), may suggest that the low insulin concentration in early lactation ensure the continuous flow of glucose to the udder, while the enhanced insulin

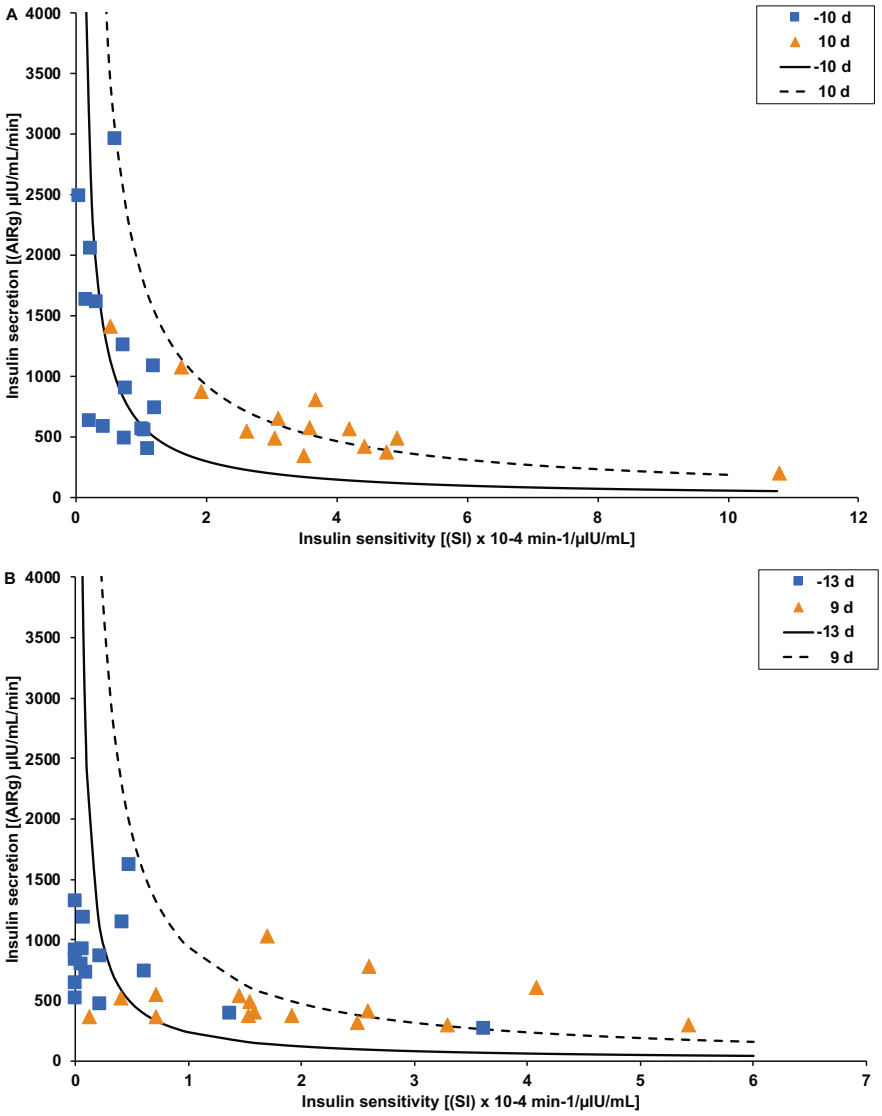

Figure 5 The hyperbolic relationship across the treatments between the minimal model– derived indices of acute insulin secretion (AIRg) and insulin sensitivity index (SI) denoted as the disposition index (DI) during the IVGTT performed in the close-up dry period (■) and shortly after parturition (▲). A: Exp. 2 (II). Treatments: CEI = 100% of ME requirements prepartum; HEI = 144% of ME requirements during weeks -6 to -4 followed by a gradual restriction of energy intake (119% of ME requirements) during the last 3 weeks of gestation. The hyperbolas were generated from extrapolated values of insulin secretion (AIR_G) based on the average of observed values of DI for −10 ± 5 (n=15) and 10 ± 1 d (n=14) relative to calving by varying SI in the range from 0.0625 to 10 (Stefanovski et al., 2011). B: Exp 3. (III). Treatments: CEI = 108% of ME requirements prepartum; HEI = 141% of ME requirements prepartum, *ad libitum* allowance. The hyperbolas were generated from extrapolated values of insulin secretion (AIRG) based on the average of observed values of DI for −13 ± 5 d (n = 16) and 9 ±1 d (n = 16) relative to calving by varying SI in the range from 0.01 to 6 (Stefanovski et al., 2011).

sensitivity and response of peripheral tissues prevent a critical deficiency of nutrient flow to the extra-mammary tissues.

Analogous hyperbola defines the relationship between basal insulin and SI (II). The curve shows that any small shifts in SI generated large changes in basal insulin in dairy cows. This implies, that when insulin sensitivity is low, small changes in insulin sensitivity would be expected to be associated with relatively large changes in fasting insulin. When SI and basal insulin values of individual cows were plotted against each other (Figure 4 in publication II) some clustered near left downward corned, whereas others were more to left and up, indicating decompensated insulin response. This data suggests that IVGTT most likely has sufficient sensitivity to identify metabolic decompensation in individual dairy cows.

The postpartal MM derived index describing the β-cell response to changes in insulin resistance, the DI across the animals in Exp. 2 and Exp. 3 (Figure 6) were higher (1856 and 945 in II and III, respectively) than those of prepartal values (412, 614 and 234 in I, II, and III, respectively). The findings may indicate that lower insulin secretory response (decreased AIRg) was sufficient to maintain and increase in the peripheral glucose tolerance as a response to improved insulin sensitivity (higher SI values; Bergman, 1989; Kahn et al., 1993; Alonso et al., 2012) after parturition (II, III). The β-cell response (AIRg) across the animals in CUDP was greater in late (II, III) than in early dry period (I). Moreover, the MM outcome variables from the IVGTT (AIRg, Sg, Si, and DI) analysis showed typical values for pregnant animals of other species, i.e. lower SI and higher AIRg to compensate for the augmented insulin resistance in late pregnancy, while SI was increased in early lactation. Hence, it may be safe to assume that the MM is capable of producing relevant estimates of whole-body insulin sensitivity in periparturient dairy cows.

Finally, given the massive need of glucose for milk production during the early lactation and the subsequent low circulating glucose and insulin concentrations, it cannot be excluded that the interpretation of the results may, at least partially, be confounded by several factors discussed in earlier chapters of this thesis. The confounding factors may create an overestimation of the MM derived index of insulin sensitivity in early lactating cows. However, in humans at least, the problems of MM estimates arise in subjects with very low insulin secretory capacity or in severe insulin resistance patients. These individuals do not show compensatory mechanisms to correct for impaired glucose tolerance during metabolic challenges (Bergman et al., 2002; Muniyappa et al., 2008), which does not correspond to current results derived during the challenges (I-III).

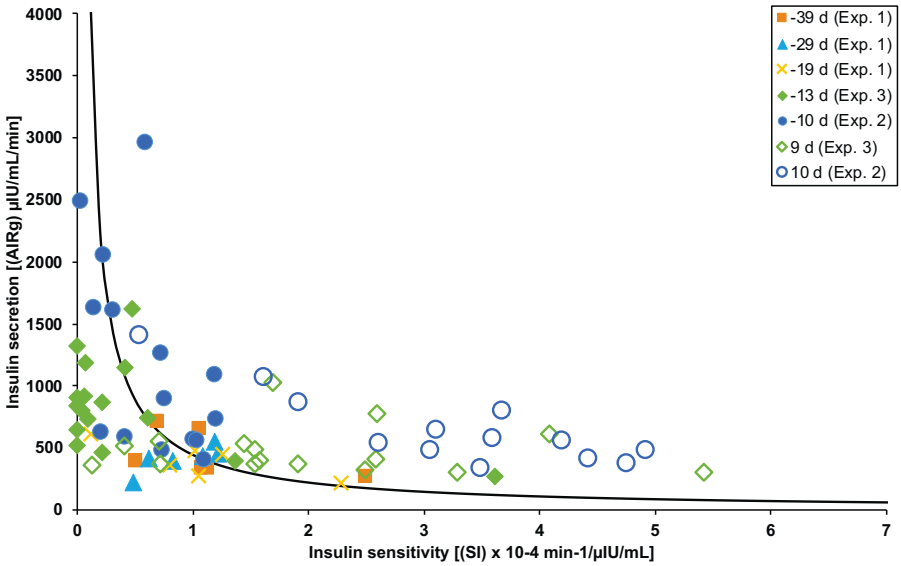

Figure 6 The hyperbolic relationship across the treatments between the minimal model– derived indices of acute insulin secretion (AIRg) and insulin sensitivity index (SI) denoted as the disposition index (DI) during the IVGTT performed in the dry period in Exp. 1-3 (I-III), and in early lactation in Exp. 2 and Exp. 3. The hyperbola was generated from extrapolated values of insulin secretion (AIRg) based on the average of observed values of DI for CON cows in Exp. 1 (n = 6) by varying SI in the range from 0.1 to 8 (Stefanovski et al., 2011). Treatments in Exp. 1 (I): CON = abomasal infusion of water (98 h); TAL = abomasal infusion of tallow (98 h); CAM = abomasal infusion of camelina oil (98 h). Treatments in Exp. 2 (II): CEI = 100% of ME requirements prepartum; HEI = 144% of ME requirements during weeks -6 to -4 followed by a gradual restriction of energy intake (119% of ME requirements) during the last 3 weeks of gestation. Treatments in Exp. 3 (III): CEI = 108% of ME requirements prepartum; HEI = 141% of ME requirements prepartum, *ad libitum* allowance.

5 CONCLUSIONS

1. Infusion of lipids, irrespective of lipid source (tallow or camelina oil) caused an increase of approximately 50% in basal plasma NEFA concentration in dry late pregnant dairy cows. The NEFA concentrations increased to a similar level than those typically observed in dry cows on grass silage-based diets during the last weeks of pregnancy. Infusion of tallow increased the proportion of saturated fatty acids of the plasma lipids, whereas camelina oil increased the proportion of 18:3n-3 when compared with tallow.
2. The induction of higher NEFA concentration by lipid infusions impaired insulin secretion and increased insulin clearance leading to diminished glucose disposal in Ayrshire dairy cows during late pregnancy. The observed responses indicated an insufficient insulin response to compensate for the deteriorated peripheral insulin sensitivity. However, infusion of camelina oil enhanced insulin sensitivity relative to tallow infusion.
3. High energy intake of moderately digestible grass silage diet during a 6 to 8-week dry period did not affect postpartal total DMI. Diluting moderately digestible grass silage with wheat straw maintained the DMI at a constant level throughout the dry period.
4. The *ad libitum* allowance of grass silage induced a moderate increment of BW and BCS gain during an 8-week dry period. In contrast, the high initial energy allowance of grass silage combined with gradual restriction of energy in the CUDP did not affect body accretion during a 6-week dry period. Prepartal energy intake did not affect mobilisation of body reserves after calving. To achieve an optimal BCS at calving, the monitoring of BCS should start already in the late lactation period.
5. The moderate negative effects of gradual restriction of prepartal energy and dilution of energy by mixing grass silage with wheat straw on early lactation milk yield demonstrated that these feeding practices were not optimal for transition dairy cows. The use of a short and scanty concentrate feeding period on TMR consisting of grass silage and wheat straw was probably not optimal for the rumen adaptation for concentrate rich lactation ration, as reflected by a slightly lower concentrate intake in early lactation. The results indicate that a higher level or a longer period of concentrate allowance should be applied prepartum in association with the type of diets.
6. High-energy diets used in the experiments of this thesis had minor negative effects on prepartal peripheral insulin resistance status as assessed by the modest deterioration of adipose tissue response to insulin. The ad libitum allowance of grass silage induced a

compensatory insulin response, preserving glucose tolerance during the prepartal IVGTT. Negligible carry-over effects of prepartal energy level on lipid and glucose metabolism were evident after parturition. The differences in energy intake between high and controlled energy diets and the dietary modifications causing alterations in glucogenic precursor availability most likely mediated the observed responses on glucose and NEFA dynamics orchestrated via insulin.

7. The findings of the thesis suggest that IVGTT with the minimal model is a useful method for the quantification of estimates of the whole-body insulin sensitivity of the transition dairy cow. The validation of minimal model derived indices in dairy cows during the transition period, however, calls for additional research.
8. The results of minimal model analysis imply that glucose and insulin dynamics during challenged conditions are governed by the hyperbolic relationship between insulin and glucose in dairy cows, as well as in other species.
9. Collectively, the data presented and discussed in this thesis suggest that peripheral insulin sensitivity of Ayrshire dairy cows is not profoundly affected by prepartal energy level on grass silage-based diets during the transition period.

6 FUTURE RESEARCH

The observations that body weight gain during the dry period induced by an ad libitum intake of grass silage leads to a compensated insulin secretion and a refractory adipose tissue to insulin in late pregnancy should be investigated further. Future researchers are challenged to identify the factors affecting inter-individual variation in the homeorhetic changes of the modern dairy cow during the transition period. It would be of high importance to assess to what extent the observed differences between individual animals are modifiable by dietary alterations.

Besides seeking to obtain the ideal composition of feeds and TMR for optimal health of the dam and support of the developing fetus and the mammary gland, further work should investigate the potential effects of overfeeding induced maternal insulin resistance on the newly born offspring. Studies in rodents and humans have shown that the normal pregnancy-induced insulin resistance is enhanced in obese mothers as reflected by increases in plasma glucose, lipids, and amino acids. The fetus may be exposed to excessive fuel supply, which in turn increases fetal size, adipose tissue stores, and risk for postnatal diseases. Indeed, it is known that offspring of obese mothers exhibit poorer glucose tolerance and disturbances in adipose tissue control of lipolysis extending over a prolonged period (Gomes et al., 2018; Zhou et al., 2020). Certainly, more in vivo studies are needed to evaluate whether fetal exposure to the metabolic overload of nutrients affects the metabolic milieu of adult cattle similarly than in monogastric species.

The lack of comprehensive data not only in IVGTT with the minimal model approach but also in HEC tests of dairy cows during the transition period may be an important gap to fill. The discrimination of the role of insulin on hepatic gluconeogenic output and that of skeletal muscle and adipose tissue uptake of glucose during the challenges conducted in different phases of the production cycle would be of crucial importance. The use of stable isotopes to measure glucose kinetics during the challenges (Muniyappa et al., 2008; Hammon et al., 2010) would give more accurate information on differences in glucose turnover between late pregnancy and early lactation, and also an estimate of the insulin resistance state of the liver (Muniyappa et al., 2008). In future approaches, the results of insulin unmodified IVGTT should be compared to values obtained from insulin modified IVGTT and those of HEC for validation of the assessment methods in dairy cows. Internal tracers of C-peptide would also be of use in determining the range of insulin degradation in the liver. Reportedly, in other animals along with the increment of systemic insulin resistance, the hepatic extraction of insulin is changed (Mittelmann et al., 2000). The obtained data should be combined with metabolomics and lipidomics approaches to get a more reliable assessment of the metabolic dynamics. The results would further serve as ground information in studies

examining the ideal nutritional approaches to support the metabolic flexibility and health and welfare of the dairy cows in the transition period.

In this thesis, it was speculated that observed differences in overconditioning induced insulin resistance during the dry period could be detected if more dramatic differences in body conditioning were evident already at dry off. To gain the targeted BCS at dry off, the treatment periods should extend beyond the dry period. Also, to control for the confounding effect of possible differences in the level of energy intake and subsequent effects on basal glucose and/or insulin concentrations at the time of the metabolic challenges, the energy intake should be equal in all dietary treatments during the week preceding the metabolic challenges.

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